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BIOLOGICAL BULLETIN

OF THE

Marine Biological Laboratory

WOODS HOLL, MASS.

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BIOLOGICAL BULLETIN.

GUESTS AND PARASITES OF THE BURROWING BEE HALICTUS.

AXEL LEONARD MELANDER AND CHARLES THOMAS BRUES.

During the months of summer every roadside presents a field of busy insect-activity, as varied and interesting as it is unseen and unheeded. Those insects, however, that we do notice are seen during their idling moments and hence we are generally accustomed to stigmatize all as idlers with no aim beyond song or frolic. But insects have a busy life — another phase of their existence which many of us overlook. If we inspect some roadside more attentively we shall be surprised to see many of the self-same idlers working with diligence. Spurred by parental anxiety these insects excavate their nests and store them with food, doing for their young what their parents have done for them.

Out of this multiplicity of insect-life we shall select as an example one of the burrowing bees of the genus *Halictus*, and endeavor to tell what may be seen on any summer day. *Halictus* (*Chloralictus*) *pruinosus* Robertson is a brilliant greenish bee, measuring about one third of an inch in length, which lives over an extended range, occurring from New Mexico, through Illinois, to Massachusetts. It is the commonest Halictine at Woods Hole, in the last-mentioned state, where the following observations were made. During the early part of summer these bees commence their excavations along the roadsides wherever a sandy slope presents a favorable situation, and continue their activities until early autumn, the colonies increasing in size, and becoming more closely settled as the season advances. They seem to be in the height of their vigor during the early part of September in this region. Although their social instincts are not so highly developed as those of *Apis* or *Bombus*, these bees depart in their habits from the strictly solitary bees in that a male and two or three females are generally necessary for the successful direction of a single ménage. Moreover, a large number of nests are usually associated as a colony which may be scattered over a considerable distance or so populous that the tunnels almost intersect by their irregularities. The openings to the nests, however, are always separated by a distance of two or three inches or more. It can thus be readily seen that *Halictus* lives under conditions more or less similar to those of their more gregarious relatives, the ants, and hence it is not surprising that they are forced to harbor the same class of guests, and to be exposed to the same vicissitudes as are their cousins.

In constructing their nests the bees dig by means of their mandibles in the sandy clay, forming a hole of a diameter only slightly greater than will admit the largest female. The wall is then banked up with a plaster formed by the aid of saliva. Immediately behind the entrance is a short blind passageway, only large enough to allow a bee to turn on itself within.

This niche, which is always less than an inch from the entrance, serves simply to allow the bees to pass one another in the interior of the nest. From this point the gallery extends nearly straight back into the hill side, for a distance of a few inches and then slopes downward to the end — a total length of a foot or so. Near the further end jut a number of small diverticula radially extending from the main tunnel.

These are the nurseries of the young bees, where are stored the pollen and honey which is destined to serve as food for the bee larvæ of the coming generation. The excavation of the tunnels is a matter of considerable toil, requiring many days for its completion, but so industriously do the little bees work that at the close of day a miniature mound of sand has accumulated on the hill-slope below the opening. During the warm portions of the day the site of each colony of nests is a scene of inspiring activity. The air is filled with an ever-changing swarm of bees, each bent on its own task of excavation or of collecting honey and pollen, while from the openings of completed nests others can be seen peering about and eying everything that comes within their range of perception. At night everything is quiet, the trespassers and robbers, too, have ceased their work, and the colony slumbers in peace.

The structure of the nest was ascertained by the ingenious plaster-cast method advocated by Prof. J. B. Smith. By this means the galleries of *Halictus* are seen to depart but little from those of the other burrowing bees. A passage-way for exit and entrance in addition to the regular one opening on the dumping ground, such as is constructed by *Augochlora humeralis* Patton,[1] was never noticed in the case of *H. pruinosus*, the vigilance required to guard two openings having probably prevented such an extravagance. All the burrows which we dug out, a dozen or so in number, extended in a nearly horizontal direction, and were always built on the very steep slopes along the roadsides. By this means none of the excavated dirt accumulated about the doorway, which was even cleared of all débris with but little effort on the part of the bee. The relatives of *pruinosus* in Texas, morphologically of the same species, select a level spot for their nesting-site, dig vertical burrows, and place the accumulated dirt in an irregular cone about the opening. A photograph of these nests is given for comparison.

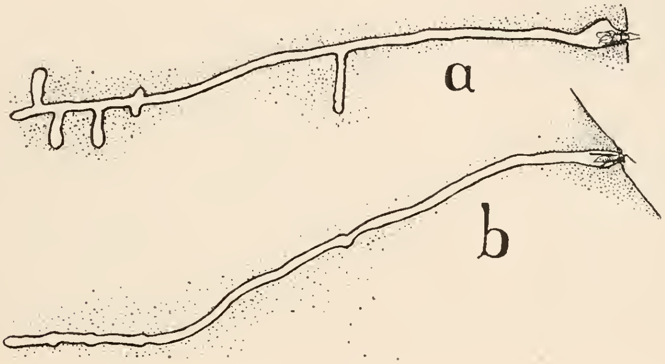

Fig. 1. Diagram of *Halictus* nest. *a*, plan ; *b*, elevation.

During the latter part of nest-construction when the pollen has been gathered and the eggs laid, their home is continually threatened by thieves and kidnappers against whom a guarded watchfulness must be maintained. The sentinels are generally the males, who sit at the doorway, their rounded heads neatly filling out the entrance. When the female returns pollen-laden, the little guard slips into the first side passage while she enters, and then as quickly returns to his post. The incomers are perceived at a distance of half a foot, probably announced by the buzzing of their wings. Even when the little watchers can not see the female coming they dart half way out of their retreat at her approach. With antennæ vibrating and mandibles spread the males either manifest a joyful greeting for their nest-mates or show an equal degree of hostility towards any stranger that may venture too near.

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[1] J. B. Smith, *Proc. Am. Ass. Adv. Sci.*, 1898, p. 368.

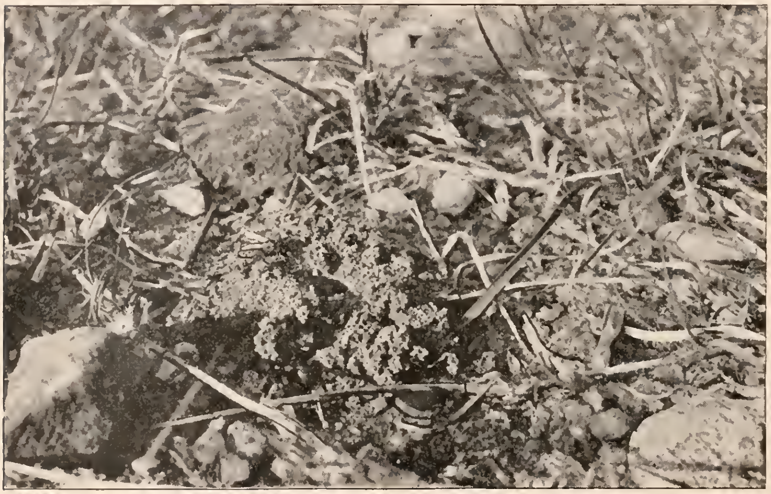

FIG. 2. Nest of *Halictus* near Austin, Texas.

The most dreaded of the enemies of the *Halicti* is perhaps the little velvet ant, *Mutilla canadensis* Blake, which is common nearly everywhere in North America, running about on the nests of these bees, its distribution practically coinciding with that of this species. Perhaps it is the stridulation produced by the abdomen of these intruders that arouses the ire of the guard at the door, for no sooner does one approach a nest than the watcher, if it be a female, rushes out and pounces upon the *Mutilla*, endeavoring to sting it to death. Down the hill-slope they roll, heedless of everything but an inborn desire to annihilate each other. The *Mutilla*, too, is armed with a powerful sting, half the length of her abdomen, but the sagacious *Halictus* grasps her enemy about the waist and most successfully evades the sharp thrusts. These combats continue for many minutes, concluded either by the invulnerable *Mutilla* slipping from the bee's grasp, for her body is hard and sleek, or by the death of the more plucky *Halictus*. Each colony, where everything seemingly is peace and content, is thus turned into a field of carnage, with the bodies of one or more females ruthlessly tumbled to the bottom of the hill. If the bee escapes unscathed, which happily is the more usual outcome of these struggles, she spends a few moments in preening her body, and then returns to her nest. But no greeting awaits her after her loyal struggle. When she hurriedly left the nest the male waiting his turn in the tunnel below quickly took her place as guard at the door, and now he blocks the entrance as obstinately as though it were a stranger begging admittance. The taint of *Mutilla* is still to be recognized on the body of the female and probably overpowers her family smell. For quite a minute she must remain at the door parleying with her mate before he is convinced of her identity.

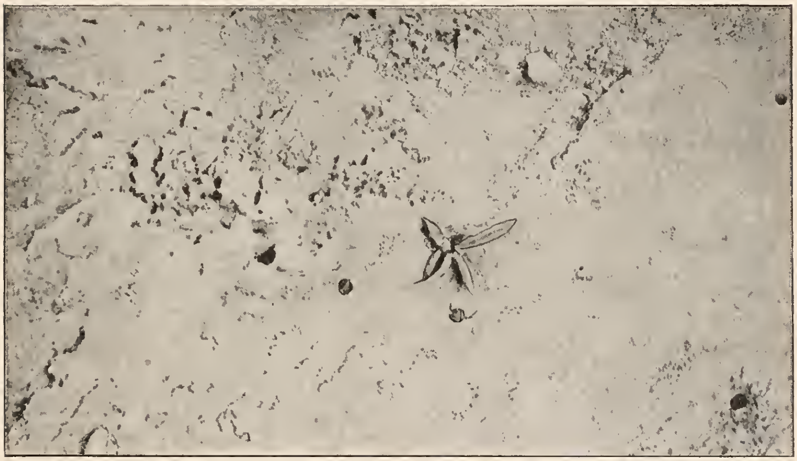

FIG. 3. Nest of *Halictus* at Woods Holl, Mass.

This observation is of interest when considering the organic dependence of instinct. Fear of *Mutilla* has been cultivated through natural selection and heredity till it manifests itself in the actions just recorded. But the conduct of the male towards his nest-mate, an inhospitable act which a gleam of reasoning intelligence would not permit under the circumstances, lends itself rather to the theory of a mechanical instinct, actuated in this case by the chemical nature of *Mutilla's* poison. If this be so it will be questioned why the bee does not behave as when *Mutilla* itself approaches. Does the mixture of *Mutilla*-influence and *Halictus*-influence compel an impassive head-on greeting while *Mutilla* alone induces the male-watcher to turn tail in the manner described on the next page?

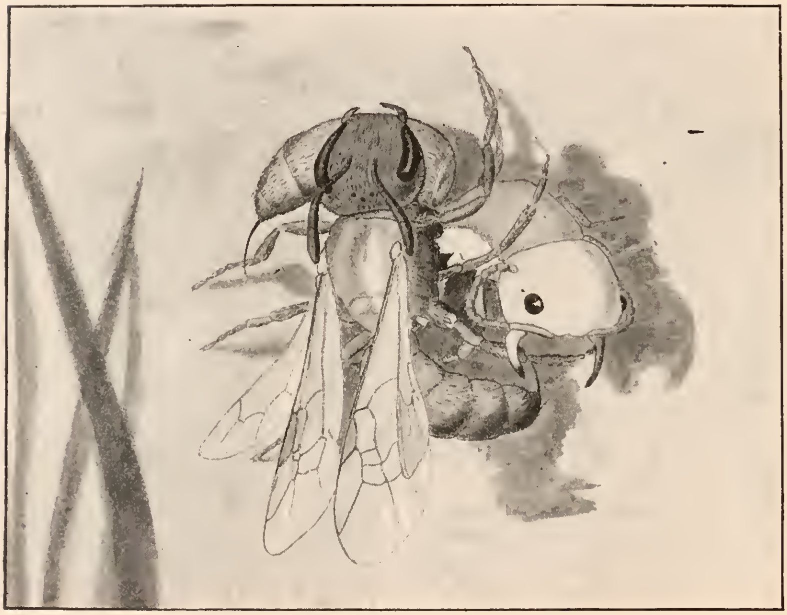

FIG. 4. Combat between *Mutilla* and *Halictus*. "Down the hill they roll heedless of everything but an inborn desire to annihilate each other."

One little bee once displayed an originality not noticed again. For fully twenty minutes she had waited at the entrance of her home, gently urging admission by advancing to the nest-opening once each minute. The male would retreat a short distance each time but not sufficiently far to admit the female, who would then retire, resting with her antennæ almost touching those of the stubborn gate-keeper. Finally she turned about and crept backward to the male, resting a moment with her sting before his face. When she now turned, the male seemed convinced, and the wearied female entered in the usual way. In this case did the female flaunt her own poison to overcome that of *Mutilla* as a passport to her home? It might seem so; but the simplicity of such a physiological action is quite equalled by the complexity of the intelligence displayed.

When a male bee guards the opening the approach of *Mutilla* produces a far different effect upon the watcher. Instead of rushing out on the marauder, the defenseless male adopts the less foolhardy measure of "turning tail," but still keeps at the entrance of the nest. Now the convex abdomen neatly fits the opening, forming a parasitic-proof shield, and *Mutilla* must needs leave. When no other bee is behind a female watcher, she never rushes out, leaving the nest unguarded, but adopts a manœuvre similar to the male's, but instead of inflexibly curving her abdomen over the opening, she reaches afar with her sting.

Canadensis, however, is not the only Mutillid that worries the Halictines. On numerous occasions *Myrmosa unicolor* Say[1] and *Mutilla infensa* sp. nov. were found crawling about, but these species do not appear to have become nearly so annoying. From one square meter of *Halictus*-colony fully fifty specimens of *canadensis* were taken during the summer, whereas in all but ten specimens of the *Myrmosa* were observed. *Mutilla ferrugata* Fabr. and *vesta* Cresson were also found prowling over the nests, though these species are doubtless parasitic on the larger burrowing insects which associate with *Halictus*, for the large size of their bodies would not permit entrance into the *Halictus* nests. Moreover, they may crawl quite close to the doorkeeper and elicit no attention; possibly their stridulation is pitched to an unresponsive key and their odor stimulates no reaction.

Almost as ardent a persecutor of the bees is to be found in a new species of *Phora*.[1] This little fly takes a stand near an opening and patiently awaits an unguarded moment. Then she quickly slips in to deposit an egg in the pollen so industriously stored. One *Phora* persisted in her attempts to enter for several hours. Driven back a half inch by the doorkeeper she gradually and slowly returned until she nearly touched his face. Then a sudden lunge half way out of the nest on the part of the bee would drive her back again. This was repeated over and over, the doggedness of the parasite and her slow approach seeming to exasperate the little watcher. By turning his head he tried to follow her movements, but from their very slowness was unable to discern her position. Only when his palpi were touched would he make a sudden dart. *Phora* depends on her agility as well as on her deliberateness. On each return of the female bee, after a fifteen-minute foraging trip, the parasite would jump about excitedly and possibly would get a chance to oviposit on the pollen mass during a dart at the bee. A moment's rest on the threshold would grant the nervous little fly ample time to infect the unsuspecting bee. The behavior of the bees towards *Phora* is quite different from the action of ants towards these guests. Unless irritated by the persistence of the parasite, *Halictus* is passive and does not notice its presence. Even the incoming females do not see the fly at a distance of half an inch. On the other hand, ants are put in a state of fright by the proximity of these flies. During the attacks of the ant-decapitating phorid, *Apocephalus Pergandei* Coq. upon the species of *Camponotus*, *ferruginea* in the north, and *maculatus* var. *sansabeanus* in the south, the ants rush in the wildest excitement with wide-spread mandibles at the agile fly. Can this difference result from the bees never seeing their offspring and being consequently unaware of their fate, whereas the ants have a personal acquaintance with the ravages of these parasites? It might seem so, but we must remember that in the case of *Pachycondyla harpax*, at least, a phorid larva is not only tolerated in the nest, but is also fed by its host.[2] In this case, however, no harm is done to the species by the presence of the fly, whereas with *Halictus* it must mean the death of the brood.

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[1] It is time to abandon superfluous names. *Myrmosa unicolor* Say, described as a male, and *M. thoracica* Blake, described as a female, have paraded in collections quite long enough as distinct species. Inasmuch as Mr. H. L. Viereck has recently taken the initiative (*Ent. News*, 1902, p. 72) in consolidating some of the species of Mutillidæ, we shall follow him in the nomenclature of this paper. The males of this species fly abundantly among the roadside flowers, in company with males of *canadensis* and *ferrugata* (= *castor* Blake = *Lepeleterii* Fox [*fenestrata* Lepeletier]).

[1] *P. halictorum*, described in the sequel.

[2] Wheeler, W. M., *Am. Nat.*, 1901, p. 1007*et seq.*

The most conspicuous of the smaller Hymenoptera that frequent these grounds is a little species of *Loxotropa.* Time and again this insect was observed crawling stealthily over the nest-colony, tapping its antennæ on the ground as it moved. During this deliberate progress it covers an inch in four seconds, but as soon as its nears a selected opening its movement slows down to an almost imperceptible advance. Still holding its long and clubbed antennæ extended straight forward, their tapping now reduced to a slight nervous vibration, it gradually insinuates itself into the nest, even beneath the very jaws of the gatekeeper. Often after crawling so far into the nest that only the tip of its abdomen is visible, it finds the nest unsuitable. Then it deliberates no longer, but makes a hasty exit, leaving the astonished sentinel to reach in vain with questioning antennæ for its bold and impudent disturber.

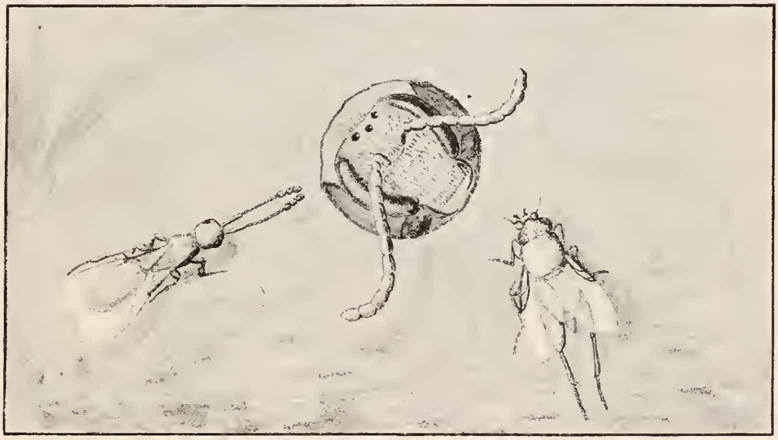

FIG. 5. *Loxotropa ruficornis* Ashm. *Halictus*, ♂. *Phora cata*, sp. nov.

As interested an observer of the incoming bees as is the *Phora*, is a tachinid fly. This species hovers over the breeding ground and suddenly circles over a particular hole. Is it attracted to the nest by the hollowness of the sound of its vibrating wings as it flies over an opening, or does it discern the state of advancement of the household below by an instinct less mechanical? Like its relatives, this species chooses the moment when the incoming bee pauses at her threshold quickly and quietly to oviposit on her pollen mass and thus infect her offspring.

A number of ants, foragers from near-by nests, are always to be found on the nesting-ground. These belong to harmless species which do not molest the bees. When an ant and a bee meet on the nest there is no encounter, each retreating good-naturedly to go her own way. The Stenammas, especially, have a stridulatory note as plaintive as that of *Mutilla*, yet this is unnoticed by the bees; even the watchers rest unaroused in their doorways while the ants pass them by. The little red thief ant is also found nesting in the midst of the bee-colony. Evidently it is here to ply its vocation of tunnelling into the chambers of the bees to steal from them their honey.

The little beetle, *Bæocera concolor*, seems quite at home with the bees. Although it belongs to a family of fungus-beetles, it, nevertheless, must have some intimate connection with the bees, as it was repeatedly observed running familiarly in and out of the nests. It is quite possible that it may live upon the pollen in deserted nests which has become mouldy by the growth of fungus hyphæ. The mixture of pollen and honey is thus readily turned into a mass of fungus under certain conditions.

The woes of the Halicti are not yet at an end. Another insect is as persevering in its depredations as its colleagues, and accomplishes by boldness what the others try by stealth. This is a larger foe, *Philanthus punctatus* by name, which audaciously builds its nest in the center of the *Halictus* colony, and when ready swoops down on a bee, stings it to death, and carries it home. Not one but many bees meet this death at the sting of their unsuspected neighbor, who plans her murders so that they take place at the flowers where the bees are at work.

When we consider the persistence of the Mutillas we can appreciate the extent to which specialization in keeping the nest parasite-proof has been carried by this bee. Seldom are the entrances left unguarded, and never is a stranger bee granted admission. In this respect *Halictus* is far more conservative than the wasp *Trypoxylon*. Although mistakes in selecting their own domicile from a cluster of fifty similar nests were frequently made, the watchers always recognized these visitors as strangers and were instantly ready to show fight. *Trypoxylon*, a wasp which also guards its doorways, on the contrary, makes no objection to the free entrance of visitors of the other sex, as has been shown by the Peckhams.[1] *Mutilla canadensis* appears to be the most dreaded enemy, as it alone is noticed by the bees. With a little reasoning ability many of the other parasites could be readily annihilated, whereas no move is made for protection against these foes except by the guard at the door. But how are the bees to know, even in the case of *Mutilla*, that their guests mean harm to their progeny? Probably they do not in a strict sense. It is evident, however, that the instinct of guarding the entrance to the nest could have been developed through the action of natural selection of favorable variations in habit, while it would be difficult to derive a number of specific reactions towards the different guests in the same manner. The very commonness of *Mutilla* and its conspicuous size are probably the reason that a specific reaction has been developed in this single case. *Halictus* is far less sensitive to its surroundings than many of the fossorial wasps are, coming and going even though we dis-

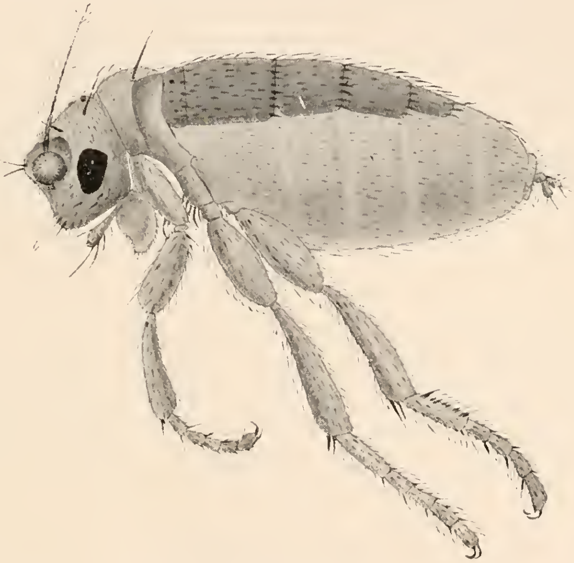

FIG. 6. *Stethopathus occidentalis*, sp. nov., lateral view.

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[1] "Instincts and Habits of the Solitary Wasps," p. 79, 1897.

turbed the nest and remained close by. Its one fear is centered in *Mutilla.* With thief-ants to rob its nests, parasites to prey on its offspring, and in constant danger of being carried away bodily by a wasp, itself numerous in individuals, it is remarkable that *Halictus* should have become a dominating type throughout such a wide territory.

This ends the list of the enemies of the bees as we have observed them. Many other insects abound on the nesting-site, but most of these, at least, are accidental visitors which neither harm nor are harmed. Several beetles, spiders, flies and other insects are included in this list which we give for reference in conclusion. The smaller species live near the *Halictus* as they would do anywhere, and not through preference, and the larger ones in part are attracted to our observation ground to prey on the smaller. These transients are such as a careful observation of any limited field would bring to notice. They are the participants in life's continual struggle, each seriously and unwittingly playing its part.

PART TWO.

A List of the Insects, Including the Accidental Visitors, Found About the Colonies of Halictus pruinosus, Robertson, at Woods Hole, Mass. July–August, 1902.

Class ARACHNIDA.

Epeirid sp.

A minute larval spider was several times seen. It has no connection with the *Halictus.*

Bathyphantes formica Emerton.

Quite a number of specimens of this strange spider were observed running in their zigzag course over the ground. Like the last it is an accidental visitor, occurring on the colony during its search for food. We are indebted to Mr. Nathan Banks for the determination of this species.

Acarina spp.

Two species of mites were obtained, one of which (*Bryobia pratensis* Garm. ?) occurred in numbers.

Class **MYRIAPODA, DIPLOPODA.**

Polyxenes fasciculatus Say.

Numerous specimens found crawling about on the sand.

Class **INSECTA.**

Order **Thysanura.**

The genus *Podura*, represented by many specimens, was found associated with the former.

Order **Hemiptera.**

Aleurodes sp.

The larval form of an Aleyrodid was discovered on the nest. Probably it is that of *A. corni* Hald., the commonest form of the Atlantic States.

Order **Diptera.**

Family CHIRONOMIDÆ.

Ceratopogon hollensis sp. nov.

Third vein in part confluent with the first, ending much beyond the middle of the wing, wings in large part hairy, not uniform in coloration, but not spotted; eyes well separated; tarsal claws simple, of an equal length; legs not spinose beneath; metatarsus much longer than the second tarsal joint.

Female. — Head fuscous, proboscis black. Antennæ fuscous, the joints uniformly moniliform, slightly longer than broad, the last two joints longer. Eyes widely separated, the front yellowish. Mesonotum pruinose, sparsely and uniformly covered with short black bristles. Abdomen dark fuscous, lightly gray pruinose, apically hairy. Pleuræ paler fuscous, smooth. Halteres dark fuscous, the stems paler. Legs slender, uniformly yellowish, except that the knees, and the tips of the femoral and tarsal joints are very narrowly black; tibiæ provided with several simple long but slender hairs on the outer edge; no bristles below, tarsi somewhat hairy, claws small, uniform, simple, empodium small. Wings sparsely covered with short bristle-like hairs, more or less serially arranged. These become obsolete at the very base, cinerascent with a pale brown tinge becoming stronger along the basal part of the course of the anterior heavy veins, gradually interrupted in front of the anterior cross-vein, then gradually recommencing to end abruptly before the tip of the first vein. The crotch of the furcation of the light vein crossing the anterior cross-vein is darkened by an accumulation of pigment and by an increase in the number of hairs.

Length, 0.85 mm.

Woods Hole, Massachusetts, August, 1902.

The nearest relative of this species is *C. variipennis* Coq.

It is not unlikely that the species is an halictophile, as it was several times seen upon the nests, thus suggesting its myrmecophilous relatives. It may also be the cause of the presence of some of the proctotrypidæ here listed, as some of them are known to prey on the larvæ of various species of the genus. This is the case with *Adeliopria* Ashm., a Diapriid, which is parasitic on a Texan species of *Ceratopogon.*[1]

Family MYCETOPHILIDÆ.

Sciara sp.

A *Sciara* would frequently fly over the nesting-site and alight on the open ground. It is an accidental visitor more at home in the nearby grass.

Family PHORIDÆ.

Phora halictorum sp. nov.

Female.—Length, 1.5–2.25 mm. Head black, subshining, antennæ black; palpi dull yellow, with stiff black bristles below; proboscis not exserted; front long, flattened, punctured, shining, its bristles reduced in size, and those of the middle row placed high up. Anterior four proclinate bristles small, the remaining ones placed normally.

Thorax black, subshining, the dorsum finely pubescent, the pleuræ lightly pruinose, ten bristles present on the hind edge of the mesonotum, dorsum with one pair of dorsocentral and four marginal scutellar bristles.

Abdomen black, shining though not brilliant, not bristly, lightly pruinose basally along the sides; ovipositor short, retractile, piceous.

Legs piceous, front legs somewhat lighter, front coxæ dull yellowish, middle and hind coxæ piceous, hind coxæ with the usual ridge on the posterior side; hind femora stoutest, twice as thick as the front ones, middle femora intermediate, all the tibiæ with short bristles, biserially arranged on their outer side, those of the front tibiæ ten to twelve in number and approximated into one line towards the inner forward edge, those of the other tibiæ in two separated series, for the middle tibiæ four in the outer and six in the inner row, and for the hind tibiæ seven in the outer and ten in the inner rows; front tibiæ without terminal spurs, middle tibiæ with one long spur three fourths the length of the metatarsus, hind tibiæ with two moderately long spurs, the outer one two-thirds as long as the inner, which is nearly as long as that of the middle tibia.

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[1] See Wm. H. Ashmead, BIOL. BULL., 1902, p. 15.

Wings hyaline with faint cinereous tinge, not brilliantly iridescent, the heavy veins nearly black, reaching very nearly to the middle of the wing. First vein but slightly bowed, third vein nearly straight, furcate, costal bristles fine and short, thickly placed, distributed as follows: four proximal to the humeral cross vein, twenty-two (double series) bordering the costal cell, ten (double series) bordering the marginal cell, and six (in double series) along the submarginal, *i. e.*, the furcation of the second heavy vein. Thin veins dark, the fourth longitudinal slightly flexed only at its extreme base, so that the cell in front is slightly wider than the one behind, ending a little closer to the wing-tip than the second light vein does, seventh vein evident, extending into the wing-margin. Halteres whitish, their stem dusky.

Male.—Length, 1.75 mm. Differs as follows: frontal bristles stouter, abdomen smaller, genitalia not distinct, small, the central filament fleshy, short, directed backward. Tibial setulæ and the inner spur of the hind tibiæ reduced in size, ridge of the hind coxæ large; costal bristles not uniform, disposed thus: prehumeral four, twenty along costal cell, four along marginal cell and four along the submarginal. The inner bristles are minute, becoming larger at the third pair of the costal cell, and from thence are much stronger than in the female.

Described from several specimens, collected as described in the previous account at Woods Hole, Mass., July–August.

This species is related to *agarici* Lintner[1] but differs by the longer bristles on the tibiæ, longer front, four scutellar bristles, etc. The habits also are quite different, as *agarici* feeds upon decaying mushrooms.

Phora rostrata sp. nov.

Female.—Length, 1.5–1.75 mm. Black, shining, legs more or less yellow, lower frontal bristles proclinate, third vein forked.

Head shining black, especially smooth and polished on the front and vertex. Front with the normal chætotaxy except that there are only two proclinate bristles at the lower edge. The front is also sparsely hairy besides the large bristles. Median longitudinal groove and ocellar tubercle unusually well-marked. Antennæ black, with a distinctly plumose arista. Proboscis piceous, very large and strongly exserted, as long as the head-height. It is slender at the base where the rather small bristly spindle-shaped black palpi are inserted, then much enlarged, swollen and bifurcated at the extremity. The bifurcation is produced by a splitting of the apex by a horizontal slit in the proboscis. Thoracic dorsum shining, hairy as usual, with one pair of dorsocentral and two scutellar bristles. Abdomen black, nowhere bristly. Legs pale yellow, the tarsi sometimes brownish ; hind tibiæ very indistinctly ciliated and with a single weak spur, as have also the middle pair. On the inner side at the apex the posterior pair have several transverse rows of short black bristles. Wings yellowish hyaline, the costal vein reaching distinctly beyond the middle of the wing and with very short cilia. First vein ending a little closer to the tip of the second than to the humeral cross vein. Fourth vein evenly arcuate, fifth vein sinuate as is also the sixth ; seventh vein present. Halteres yellowish, blackened at the tips.

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[1] 10th N. Y. Rept., pp. 399–406.

Described from two female specimens collected at Woods Hole, Mass., July 15, 1902, about the burrows of *Halictus pruinosus.*

This species is readily recognizable on account of the excessive development of the proboscis, which is evidently adapted to some peculiar method of food-getting. It is also characterized especially by the very shining front, which seems to place it near to the European *P. minor* Zett., with which it agrees is some other characters.

Phora cata sp. nov.

Male and Female. — 0.8–1.2 mm. Black, legs and palpi yellowish or brown, antennæ of male enlarged. Anterior frontal bristles proclinate.

Head black, front short, about as wide as long, subshining, faintly gray pollinose in the male, two anterior bristles proclinate, the others all present and arranged as usual. Antennæ wholly black in the male, in which sex the third joint is enlarged and ovate so as to be very conspicuous, in the female they are of the usual size and slightly yellowish at the base ; arista pubescent. Palpi light yellow, strongly bristly. Proboscis of female projecting, stout and horny. Thorax shining, black, hairy, with one pair of dorsocentrals and two marginal scutellar bristles. Abdomen black. Legs yellowish-brown, the anterior pair lighter. Posterior femora ciliated below on apical half, their tibiæ without any rows of small bristles on the outer side ; four posterior tibiæ each with a delicate apical spur. Wings hyaline, the costal vein not quite reaching to the middle of the wing, its cilia short and closely placed. Third vein far from the costa at its base, and forked very near the apex. Tip of first vein twice as far from the humeral vein as from the tip of the second. Fourth vein slightly but evenly curved, recurved at the extreme tip. Fifth slightly diverging from the fourth to its tip, which is as far behind the wing tip as the fourth is before it. Seventh vein faint but distinct. Halteres yellowish in the female, piceous in the male.

Described from a single pair from Woods Hole, Mass. The lighter color of the female is most likely due to her apparently immature condition. They were taken on the sand in the midst of a colony of *Halictus.*

This species can readily be recognized in the male sex by the enlarged third joint of the antenna. The female is not so characteristic, but can be distinguished by the combination of structural characters given in the description. It resembles most closely *P. agarici* Lintner, but has very short costal bristles.

Stethopathus Wand.

Among the insects frequenting the ground immediately about the *Halictus*-burrows was one extremely small form, which from its quick motions we immediately suspected to be a wingless phorid fly. Such it indeed proved to be, but of quite a different sort from any of our previously discovered North American species. Its occurrence in New England is quite unexpected and considerably extends the range of such forms, as none have hitherto been seen in America north of central Texas.

Its associations with the *Halictus* may be doubtful, although no specimens could be found elsewhere whereas three females were captured where the burrows of the bees were abundant. Nests of *Lasius niger* and of *Stenamma fulvum*, variety *piceum* also abound in such locations, but close scrutiny of the ant nests revealed no specimens of the Phoridæ. The fact that species of *Phora* occur as parasites of these bees would make it seem not improbable that the *Stethopathus* has similar habits. We have also a single winged male phorid, captured at the same time, but which is probably the male of some other undescribed form on account of its larger size and the different chætotaxy of the head. The description of this interesting little wingless fly, one of the smallest known of all the Diptera, is given herewith.

Stethopathus occidentalis sp. nov.

Female.—Head rounded triangular, much rounded on the sides and at the hind angles and obtusely pointed in front, about two thirds as long as wide above, vertex descending rather steeply and evenly. Eyes small, about one and one third times as large as the second antennal joint, coarsely facetted with hemispherical ommatidia as usual. Antennæ placed at the bottom of the deep frontal cavities. Proboscis long and stout, equal to the head-height ; palpi small and slender, thickest near the tips, with stout macrochætæ on the inner side. Ocelli present, placed in a small triangle on the vertex. Head with four closely approximated macrochætæ at the middle of the front margin, two widely-separated ones near the anterior corner of the eye directed inwards and two outwardly directed ones near the posterior angles ; a series of small macrochætæ below and in front of the eye.

Thorax small, twice as wide as long, truncate before and behind ; sinuate on the sides and narrowed behind, so that the pleuræ are slightly visible from above. Thorax rather sharply arched above, and much narrowed below on the sides. Dorsal surface with a pair of long macrochætæ just behind the anterior angles and four smaller marginal ones along the posterior edge.

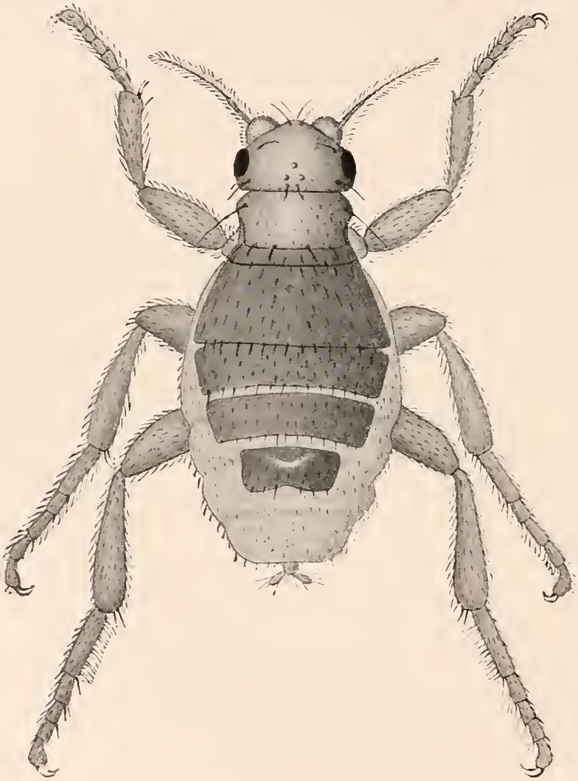

FIG. 7. *Stethopathus occidentalis* sp. nov., dorsal view.

Abdomen considerably swollen, but with large and strongly chitinized dorsal plates. The first is only a narrow band, contiguous with the second which is very large and contiguous with the third. The fourth and fifth are separated by a white membrane such as covers the abdomen elsewhere. Seen from above the abdomen is twice as wide as the thorax and flattened, oval in cross-section. No ventral sclerites are present. Each segment is margined behind with small bristles and is hairy elsewhere as is the entire body. Glandular opening of the fifth segment[1] in the shape of an arcuate slit. External genital organs of the usual form. Legs rather stout, the tibiæ with two apical spurs.

Length, 0.75 mm. Testaceous, head and thorax darker above, especially directly about the ocelli. Abdominal plates dark fuscous, the membranous parts almost white, with a small fuscous spot at the insertion of each hair.

This form is a typical representative of the Stethopathinæ and strange to say, it approaches more nearly to the East Indian *Stethopathus ocellatus* Wand. than to any of the species that have hitherto been discovered in America. Indeed, it is here regarded as congeneric with the former, although the two species are from such widely separated regions of the earth.[2] It may be necessary later to separate these two forms, but at the present state of our knowledge of this group it does not seem advisable. The American species resembles *S. ocellatus* Wand. in possessing ocelli, being utterly destitute of wings and halteres, and in having a similarly shaped head and abdomen. But differences in form are also evident: the thorax is only twice as wide as long, instead of three times as in *ocellatus*, the palpi are clavate, not spindle-shaped, and the chætotaxy is somewhat different, although conforming to the same general type. Although its habitus seems to be quite different from that of the genus *Ænigmatias* Meinert (which it may be recalled, has just been discovered in Arizona,[3] a locality quite distant from its home in Denmark), yet this species may possibly prove to be a close relative.

A point perhaps of minor importance, but nevertheless interesting as bearing upon its systematic position, is the fact that the

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[1] In previous papers the gland opening has been referred to the fourth segment of the abdomen, but the very short first segment in the present species leads us to believe that this sclerite is concealed in the other American species and that there, too, the gland really opens on the fifth segment.

[2] Many cases might be mentioned of monotypical or very small genera of insects which have an inexplicably wide discontinuous distribution. *Amphizoa* with two species, one in western North America and another in Tibet; *Syntelia*, which is represented by two species, occurs in Mexico and eastern Asia; and the water-beetle, *Pelobius*, occurring in western Europe, Tibet and Australia. For further references to the close approximation in certain details of the faunæ of eastern North America and Asia, see C. C. Adams, "The Southeastern United States as a Center of Geographical Distribution of Flora and Fauna," BIOL. BULL., Vol. III., pp. 115, et seq.

[3] D. W. Coquillett, *Can. Ent.*, 1903, p. 20.

American species has, like the East Indian form, bare, non-pubescent macrochætæ, while the other American species of this subfamily have them pubescent.

Family TACHINIDÆ.

Metopia leucocephala Rossi.

The interested observer of the *Halictus* mentioned in the first part was captured for identification, and proves to belong to this widely distributed species.

Order **Hymenoptera**.

Family BRACONIDÆ.

Subfamily CHELONINÆ.

Chelonus brevipennis sp. nov.

Female.—Length, 2 mm. Ferruginous, head piceous black, wings reaching just beyond the base of the abdomen.[1] Antennæ 21-jointed, tapering as usual, and almost as long as the body, ferruginous at the base, black at the tip, the third joint four times as long as thick, the apical joints more or less quadrate-moniliform. Eyes smaller and less densely hairy than usual. Head almost smooth above, shining, thinly pale pubescent, piceous black above, ferruginous below, palpi yellow. Thorax ferruginous, pronotum coarsely rugose reticulate above, mesonotum less distinctly so, metanotum small, quadrate, not toothed at the posterior angles, rugoso-reticulate ; pleuræ not roughly sculptured, somewhat shining. Abdomen with no traces of sutures above, dark ferruginous and sparsely white hairy ; gradually broadened from the base and rounded at apex ; finely and irregularly reticulately striate longitudinally, especially at the base. The incurved margin is emarginate at the apex of the abdomen. Ovipositor stout, black. Legs long and slender, yellow, the femora clavate.

Described from a single female specimen collected at Woods Hole, Mass., in a burrow of *Halictus pruinosus*.

The present species seems best referable to *Chelonus* because of its pubescent eyes. The apex of the abdomen however is emarginate, somewhat as in *Gastrotheca* Guérin. Unfortunately as the wings are rudimentary they can not be used to determine its affinities. The only other apterous species belonging to this subfamily are included in *Acampsis* Wesmael, from which the present form differs by its unsegmented abdomen.

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[1] For neuration, see BIOL. BULL., 1903, p. 189, Fig. 5.

Family CHALCIDIDÆ.[1]

Eupelmus rhizophelus Ashmead.[2]

This remarkable chalcidid with vestigial wings in the female was seen rather commonly about the *Halictus* burrows. As it has been previously bred from cynipid root galls by Mr. Ashmead, it is no doubt an accidental visitor to the bee nests.

Eupelmus Ashmeadii sp. nov.

Female. — Length, 3.5–4 mm., ovipositor 0.5 mm. Shining green varied with ferruginous on the thorax and with luteous and black on the abdomen. Head shining green, with a sparse white pubescence. Mandibles brown, black at the tips, palpi black. Antennæ long, the scape yellow, reaching to the ocelli, flagellum black, about once and one half the head-height, last joint acutely pointed. Head less than twice as wide as long, the space between the eyes above narrow, so that the lateral ocelli are close to the eye-margin. Face rugoso-punctate with a median carina extending from the clypeus to the insertion of the antennæ. Prothorax shining brown. Mesonotum very closely punctate, not at all shining, brown in front and green behind, concave medially behind, on each side of the depression it is raised and almost carinate, then slopes down to the reflexed margin ; anteriorly it is raised to form a broad triangular tubercle. Pleuræ ferruginous except in front where they are green. Metanotum golden, closely punctate, bilobed, sharply declivous, forming a right angle with the mesonotum. Wings deeply infuscated, paler at base and slightly so at apex, with a narrow cross band of white just before the stigmal vein. Marginal vein equal to one third the length of the wing, stigmal vein moderate, one half the length of the post-marginal. Abdomen shining black, pale luteous on the basal third. Sheaths of the ovipositor bright ferruginous, almost as long as the abdomen. Legs brown, darker on the front and hind femora, tarsi yellowish except the tips.

Described from three female specimens collected at Woods Hole, Mass., July and August, 1902.

This pretty species was associated with the much smaller brachypterous species, *Eupelmus rhizophelus* Ashm., on the burrows of *Halictus pruinosus.* It is named in honor of Mr. Wm. H. Ashmead, who determined it as an undescribed species.

Henicopygus subapterus Ashmead.

We have seen this species running actively about on the ground among *Halictus* burrows at Austin, Texas. Like the species of *Eupelmus*, it may be an accidental visitor.

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[1] We are indebted to Mr. Wm. H. Ashmead for his kindness in determining the species of Chalcididæ.

[2] For wing-neuration, see BIOL. BULL., 1903, p. 189, Fig. 7.

Encyrtinæ gen. et sp. indesc.

Among the Chalcididæ there is a single specimen which Mr. Ashmead, who has kindly examined it, informs us represents an undescribed genus of Encyrtinæ. Unfortunately it is too poorly preserved to permit of an accurate characterization in the large and difficult group to which it belongs.

Cirrospiloideus (Miotropis) platynotæ Howard.

A single female of this species was captured.

Superfamily PROCTOTRYPOIDEA.

Family Scelionidæ.

Telenomus sp.

There is a single pair representing an apparently undescribed species in this large and difficult genus.

Caloteleia Marlattii Ashmead.

This active little species is a regular visitor about the nests.

Caloteleia parvipennis sp. nov.

Female. — Length, 2.5 mm. Yellow, varied with darker. Head black, very smooth and polished above the antennæ, finely punctured on the vertex and with larger punctures intermixed. Mandibles yellow at the base, black at the tip. Antennal scape pale yellow, reaching a little above the vertex, the pedicel small and rounded, yellow, the flagellum about one and one half times the length on the scape, black, the first flagellar joint twice as long as the pedicel, then the joints decrease in size to the fourth, the following six forming a thick oval club with closely articulated joints. Thorax entirely yellow, except the tegulæ which are black, mesonotum finely punctulate, with two rather faintly marked furrows, scutellum large, semicircular, smooth. Metathorax very short, emarginate in the middle, smooth on the sides. Abdomen polished and perfectly smooth, except for coarse longitudinal striæ on the first and at the base of the second segments. The petiole is short quadrate, and bears a quite distinct polished black tubercular horn at its base ; basal half of abdomen otherwise yellowish varied with brown, apical half black ; third segment longest, second nearly as long, others much shorter. Legs including the coxæ yellow. Wings short, reaching only to the middle of the abdomen. Marginal vein short and swollen, stigmal about one third as long as the lengthened postmarginal, costal margin sparsely ciliated.

Described from one female specimen taken at Woods Hole, Mass., on a slope that was thickly riddled with the burrows of

Halictus. When captured it made no attempt to fly, the wings evidently being too much atrophied to be of functional use.

This form can be readily recognized by its short wings. It does not seem to be very closely related to any of the other North American species.

Scelio ovivorus Riley.

This large and coarsely sculptured Scelionid was originally bred by Scudder from the eggs of the common New England grasshopper (*Dissosteira carolina*) so that its occurrence is evidently not connected with the presence of the *Halictus* colony. Nevertheless it was often seen intermingling with the bees.

Family DIAPRIIDÆ.

Loxotropa ruficornis Ashmead.

This is a common species always to be found on the breeding ground of these bees. Its habits have already been noted in the preceding part of this paper.

Family BETHYLIDÆ.

Empyris subapterus sp. nov.

Female.—Length, 3.25 mm. Black, head and thorax subopaque, abdomen shining; antennæ, mandibles at tips, palpi, tegulæ and extreme tip of abdomen rufous; sparsely pale pubescent. Head about one third longer than wide, closely and finely punctate with fewer larger punctures intermixed. Antennæ reaching about to the tegulæ, scape stout and curved, three times as long as its thickness at the tip; following joints of about equal length, except the first flagellar, which is shorter; pedicel more slender, the other joints slightly wider than long. Eyes hairy, ocelli present. Prothorax sculptured like the head, with a transverse impressed line anteriorly. Mesonotum very short, less than half as long as wide, without grooves or furrows. Tegulæ rufous. Scutellum basally with a deep transverse linear fovea. Metanotum about one and one half times as long as wide, with a median longitudinal carina and a fainter one close on each side of it anteriorly, also a lateral and an apical carina present; surface elsewhere finely transversely rugulose; posterior face sharply declivous, shining and punctulate. Wings abbreviated,[1] just attaining the apex of the metanotum; with a small stigma near the apex, a narrow, submarginal cell and an equally long but wider basal cell; costal margin fringed. Legs, including the coxæ, dull rufous. Abdomen polished black, the margin of the penultimate segment and the apical half of the last segment ferruginous.

[1] For figure see BIOL. BULL., 1903, p. 189, Fig. 2.

Described from several female specimens collected at Woods Holl, Mass., running about among the burrows of a colony of *Halictus pruinosus* Robts.

This species greatly resembles *Mesitius* in habitus, but has a transverse furrow at the base of the scutellum instead of two foveæ. It can hardly be the undescribed female of *E. carbonarius* Ashmead, on account of the difference in the sculpture of the metanotum. It is apparently the first subapterous form to be described in this genus.

Family FORMICIDÆ.

Lasius niger Linneus.

Stenamma fulvum var. **piceum** Buckley.

Solenopsis molesta Say.

This last named species is the only one that derives any direct benefit from the presence of the bees.

Family MUTILLIDÆ.

Mutilla canadensis Blake.

This is the most conspicuous of the enemies of the bees. It has been fully noticed in the preceding part.

Mutilla infensa sp. nov.

Female.—Clothed with sparse appressed white pubescence becoming denser apically, and with scattered long erect hairs. The hairs are black on the vertex, dorsulum and second abdominal segment and become whitish on the under side of the body and beyond the second segment of the abdomen. Coarsely sculptured species ; head finely and closely punctate, thorax and petiole of the abdomen coarsely reticulate, abdomen much less deeply and more distantly punctured than the head, the apical segments with finer punctures, meso and metapleuræ shining, not or but little strigose, nearly smooth, pygidium longitudinally closely but irregularly striated, the striæ very weak and vanishing apically. Head quadrate, concave behind, in profile also rounded ; eyes prominent, round, subshining, their facets distinct ; mandibles straight, strong, pointed, untoothed ; scape stout, as long as the three basal joints of the flagellum, basal flagellar joints subequal. Thorax elongate-oval, nearly as broad as the head, the front margin and angles well defined, posterior surface of the metanotum not sharply declivous, somewhat flattened and rounded above. Petiole of the abdomen flattened above, constricted from the second segment, one fourth broader than long, its front angles sharp and prominent, its ventral carina weak, very obtusely angulate at the middle and minutely toothed in front. Legs slender, provided like the body with silvery erect hairs, four or more strong spines on the outer edge of the hind tibiæ, the tibial spurs and spines black.

Ferruginous or somewhat darker, the mandibles, the flagellum except its basal joint, *i. e.*, the third antennal joint, more or less of the second abdominal segment, and all of the other segments of the abdomen, from the third apically, both ventrally and dorsally black. Legs including the coxæ piceous or black. Second segment of the abdomen with a varying extent of the front margin, a diffused median vitta and the hind margin more strongly black or blackish. On each side of the median stripe is a pair of conspicuous rounded testaceous spots. Last ventral segment sometimes reddish.

Length, 4.75 mm.

Woods Hole, Massachusetts. Parasitic on *Halictus pruinosus* (*?*).

The edentate mandibles, the facetted eyes and the nodose petiole of the abdomen would lead one in placing this species in the small group *scrupea*, where it is obviously distinct from the only other known female by its rugose thorax, etc. Notwithstanding this, we shall have to disregard the well-marked ommatidia and place the species in the group *occidentalis*, intermediate between *cariniceps* Fox and *rugulosa* Fox, differing from each by the structure of the pygidium, etc., but related by its general habitus, sculpture and chætotaxy.

Mutilla vesta Cresson.

Mutilla ferrugata Fabricius.

Like the former species this too is doubtless parasitic on the larger Hymenoptera such as *Philanthus* or the Pompilidæ that nest near by.[1]

Myrmosa unicolor Say.

The males of this species fly about the roadside flowers while the females are frequently found about the bee nests. Their presence is undoubtedly due to the bees.

Family PHILANTHIDÆ.

Philantus punctatus Say.

This species was observed nesting in the very midst of several of the colonies of *Halictus*.

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[1] In Europe Sichel records *M. incompleta* Lep. as parasitic on *Halictus* (cf. Horæ, *Soc. ent. Ross.*, VI., p. 11) and *M. coronata* as a parasite of *Larra anathema* (*ibid.*, p. 12).

Sphex ichneumonea Linn., and a species of Pompilidæ were also seen digging their nests in the compact sand of the road in the vicinity of the bee colony. They have no connection with the presence of the bees, but associate with them as the same condition of soil and surroundings are suitable for each.

Order **Coleoptera.**

Family COCCINELLIDÆ.

Microweisea misella Leconte.[1]

The species of this genus are reported to be of great economic importance as they greedily prey on scale insects. The presence of the *Aleyrodes* may have had an influence in bringing this species to our notice.

Family ENDOMYCHIDÆ.

Aphorista vittata Fabricius.

Family PTINIDÆ.

Cænocara scymnoides Leconte.

Family SCAPHIDIDÆ.

Bæocera concolor Fabricius.

The last three species are fungus-eating beetles, which may come to the *Halictus* nests to feed on the fungus overgrowing the stores of abandoned or damp nests. It is certain that during the course of the season numerous nests are left unfinished, either deserted voluntarily by the bees for some whimsical reason or not completed by the death of the bees.

Family RHIPIPHORIDÆ.

Myodites fasciatus Say.

Inasmuch as Fabre and others have found the larvæ and pupæ of a member of this family in the cells of an European species of *Halictus*, it is quite interesting to note the occurrence of *M. fasciatus* about the colonies of the American form. Several specimens were taken while sweeping with the net among the swarming bees as they entered and left their nests.

Several other beetles were found crawling over the nest but were visitants too accidental to record.

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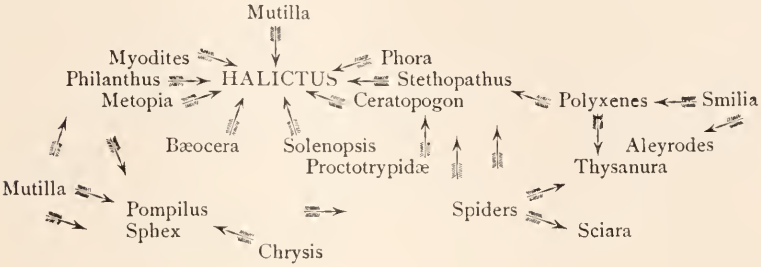
[1] This is the species known in our lists under the generic name *Smilia* or *Pentilia*. The present name was proposed by Cockerell (*Can. Ent.*, 1903, p. 38).

In conclusion we may present the following diagram showing the interrelationships of the most important of the insects we have observed. For *Halictus* it is indeed a "whirlpool of life" with only too many vortices centered upon its unfortunate self.


Mutilla
Myodites
Phora
Philanthus
HALICTUS
Stethopathus
Metopia
Ceratopogon
Polyxenes
Smilia
Bæocera
Solenopsis
Aleyrodes
Proctotrypidæ
Thysanura
Mutilla
Pompilus
Sphex
Spiders
Sciara
Chrysis

THE ORIGIN OF THE HEART ENDOTHELIUM IN AMPHIBIA.[1]

J. B. JOHNSTON.

The origin of the heart endothelium in Amphibia has been the subject of several special investigations and of a considerable volume of discussion. The question of fact may now be regarded as settled. The work of Brachet has given definite and conclusive evidence that the endothelium is derived directly from the entoblast, as had been shown to be very probable by the earlier work of Rabl and Schwink. The question now of interest is, how is the derivation of the heart endothelium from the entoblast in amphibia to be harmonized with its known origin from the mesoblast in all other vertebrates? The problem is that of the homology of the heart endothelium of amphibia. Granted that, as Ziegler contends, the condition in amphibia is to be regarded as the result of cœnogenetic modification, exactly what is the modification that has taken place? What is the definite explanation of the striking difference between amphibia and other vertebrates? As Brachet has pointed out, the term "cœnogenesis" can not be invoked as a magic symbol to dispense with the whole matter. It is not enough to say that in amphibia the endothelial cells remain connected with the entoblast until a late period and become separated after the mesoblast sheet has split off. This offers no escape from the difficulty pointed out by Morgan ('97, p. 151) that the heart endothelium must be considered to have a different origin from the rest of the heart.

The work upon which the present paper is based has been done upon the eggs of a salamander which have been used for class study for the past two years. The species has not been identified because no adults have yet been taken. I hope at some later time to give a description of these eggs and to deal with some other features of the embryology of the species. The eggs have proved very favorable for study and the facts are so clearly made out that they are thought to offer a solution of the problem.

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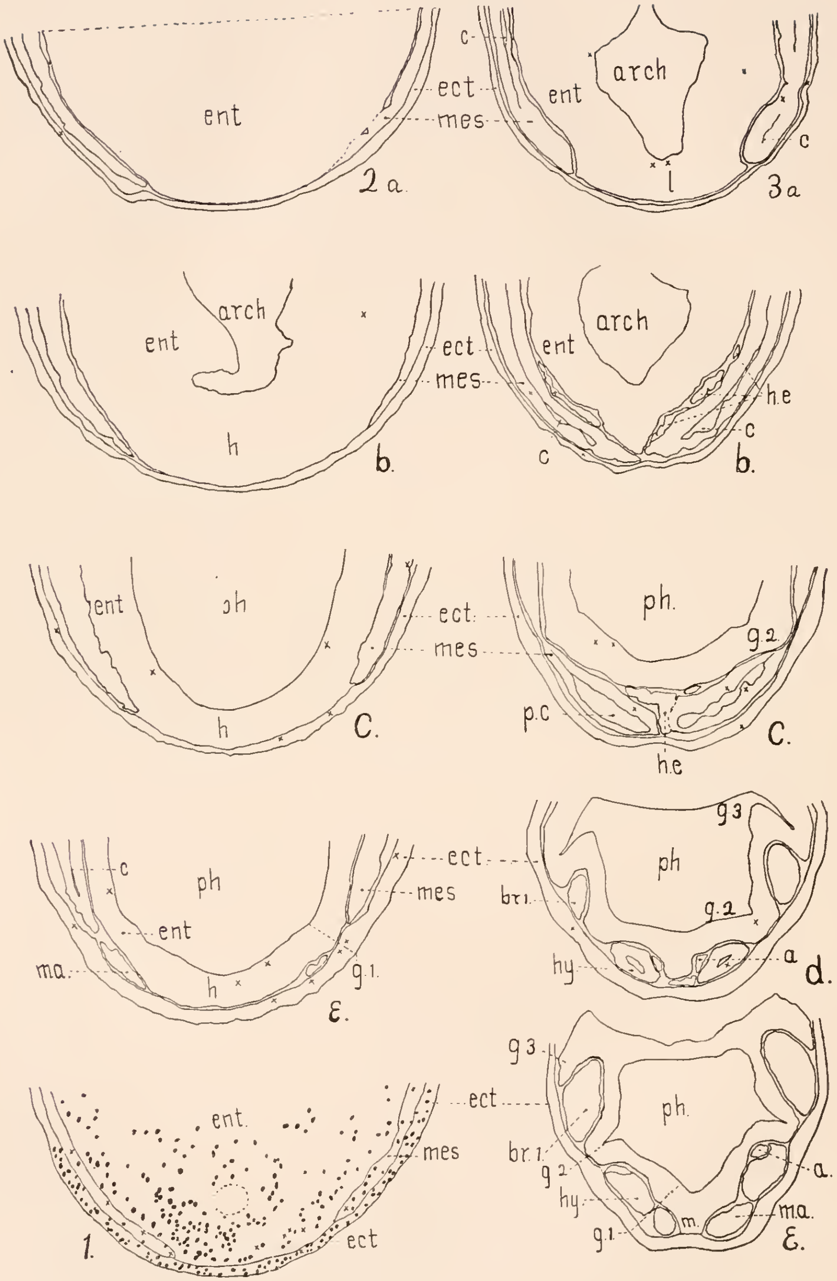
[1] Studies from the Zoölogical Laboratory of West Virginia University, No. 7 February 27, 1903.

The earliest indication of the formation of the heart endothelium is found in the rapid multiplication of the cells of the entoblast just behind the mouth anlage, at a period when the head is slightly turned downward and before the gill slits have begun to appear. As shown in Fig. 1, the nuclei in this part of the entoblast are small, rounded, very numerous and closely crowded, and many of them are in some stage of mitosis. The nuclei in the remainder of the entoblast are larger and irregular, being much distorted by pressure of the yolk grains, and mitotic figures are rare. The area described extends for a considerable distance backward from the mouth, and the same conditions prevail on the cephalic surface and the sides of the pharynx close to the mouth anlage. Rapid growth in these latter regions continues later than behind the mouth and is connected with the formation of head mesenchyme. The region of growth behind the mouth is noticeable in both transverse and sagittal sections, but it is of short duration and in slightly later stages the cells are relatively larger and the nuclei have the appearance of resting nuclei. At the point nearest the mouth the cell divisions continue until the time of separation of the heart endothelium.

The formation of the mesoblast and its early differentiation furnish the facts of greatest significance for our problem. In the head and anterior part of the trunk the mesoblast is split off from the entoblast to a point some distance from the mid-ventral line, where the delamination appears to stop. That this is a definite limit beyond which delamination does not go is evidenced by the distinct separation between entoblast and mesoblast which often occurs even in very early stages (Fig. 2, *a* and *b*), by the total absence of nuclei in the outer half of the entoblast ventral to the limit mentioned, and by the future history of the ventral portion of the mesoblast. Mitotic figures often appear very early in the ventral edge of the mesoblast sheet (Fig. 1), and although they do not appear in the sections drawn in Fig. 2, they are usually more numerous there than elsewhere in the mesoblast. The result of rapid growth here is to cause a decided thickening of the ventral edge of the mesoblast, and in this thickening the body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Figs. 1, 2 and 3.

cavity early makes its appearance (Fig. 3, *a*). With further growth the body cavity enlarges and the entoblast is laterally compressed between the cavities of the two sides. As a result, the growing entoblast behind the mouth, above described, takes on the form of a keel. Later the body cavity (pericardial cavity) spreads ventrally and mesially, and the mesoblast insinuates itself between the heart endothelial cells and the ectoblast and later between these cells and the entoblast. This movement is due entirely to the growth and spreading of the mesoblast earlier split off and not to a further delamination from the entoblast. There is no sign of any further delamination of mesoblast after the stage shown in Fig. 2, but on the contrary the mesoblast grows continually more and more sharply distinct from the entoblast after that period. The pushing down of the mesoblast in the region of the heart, which accompanies the enlargement of the pericardial cavities, is well advanced while the thickened ventral édge of the mesoblast farther caudally has not shifted its position (Fig. 3, *a*, *b*, *c*). The region in which the delamination of mesoblast does not reach the mid-ventral line extends caudally to a point a little behind the middle of the embryo and this region probably includes the blood island described by Brachet. The writer has not yet fully investigated this region, but if the surmise here made is correct, the reasoning applied to the question of the heart endothelium will apply equally well to the blood island. To recapitulate, there is a mid-ventral area or keel of entoblast extending backward from the mouth anlage, from which no mesoblast is split off in the species studied. From this area the heart endothelium (and perhaps the blood) are formed.

A second fact of some interest for us is that the mesoblast shows a tendency to split off late, so that it is already divided into regions when it first separates from the entoblast. This is seen especially in the formation of the mandibular arch. As shown by Fig. 2, *c*, the mandibular arch mesoblast, at its first appearance is separated from the rest of the mesoblast by the first gill slit, and it never has any connection with the mesoblast bounding the pericardial cavity. Indications of the second gill slit also appear very early, so that in some cases the hyoid arch, which is continuous with the pericardial cavity, seems to be split off from the entoblast separately from the rest of the mesoblast. Finally, single cells wander off from the cephalic surface of the entoblast and go immediately to the formation of head mesenchyme.

The mode of formation of the heart endothelium from the ventral keel of entoblast differs in details in different forms. In the Urodeles studied by Brachet, the keel of entoblast extending from the mouth anlage to the region of the liver splits off as a continuous rod, the cells of which later arrange themselves into a tube. In the species studied by the writer the cells of this keel do not remain in a continuous rod but split off singly or in groups of a few cells and form a loose mass which remains connected with the entoblast longest at the end nearest the mouth. At this point there is continued growth and there is probably a migration of cells from this point backward, and also upward into the several branchial arches, to form the aortic arches. The splitting off of this keel of entoblast is taking place simultaneously with the spreading ventrally and mesially of the pericardial mesoblast. My preparations leave no doubt whatever that the heart endothelium is formed from the most superficial portion of the entoblast in the mid-ventral region and that the lateral sheets of mesoblast are formed wholly outside of this area. Brachet's description makes it clear that the same thing is true of the Urodeles which he studied, but this important relation seems not to have attracted his attention and the fact is not mentioned by him.

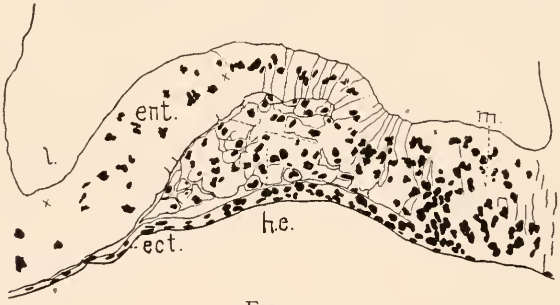

FIG. 4.

We are now able to state definitely the nature of the cœnogenetic modifications connected with the formation of the heart endothelium in amphibia. According to the earlier accounts which have recognized the derivation of the endothelium directly from the entoblast, the mesoblast sheets were split off first, and later — consequently from deeper layers of entoblast — the cells destined to form the heart endothelium were split off. Since in other vertebrates only one layer of cells is split off and the heart endothelium is differentiated from a part of this mesoblast, the conclusion that the endothelium of amphibia has a different origin from that of other vertebrates was unanswerable. In the species studied by the writer (and also, apparently, in those described by Brachet) the mesoblast sheets are split off earlier from the entoblast except in the region in which the heart endothelium will appear, and later the endothelium is split off from a part of the entoblast which has not given rise to any (other) mesoblast. Therefore, in these forms, the heart endothelium is derived from the same source as the mesoblast sheets, namely from the superficial layer of entoblast, and the difference between these amphibia and other classes of vertebrates consists only in a somewhat general tendency for the mesoblast to split off relatively late and to be marked out into definite organs, or organ-anlages, at the moment of splitting off. This is seen not only in the splitting off of the heart endothelium at a little later time and separately from the rest of the mesoblast, but also in the same mode of formation of the mandibular and hyoid arches and of a part of the head mesenchyme. The writer believes that a reëxamination of other amphibia, at least of Urodeles, at the proper stages of development will show the process here described to be characteristic for amphibia. In brief, then, the heart endothelium of amphibia is strictly mesoblastic, although it is not at any stage identified with the undifferentiated mesoblast, being split off from the entoblast in the same manner as the rest of the mesoblast, but somewhat later and separately.

DESCRIPTION OF FIGURES. ABBREVIATIONS.

a., aortic arch cells ; *arch.*, archenteron ; *br.1*, first branchial arch ; *c.*, cœlome ; *ect.*, ectoblast ; *ent.*, entoblast ; *g.1*, *g.2*, *g.3*, first, second, and third gill slits ; *h.*, heart region ; *h.e.*, heart endothelium ; *hy.*, hyoid arch ; *l.*, liver region ; *m.*, site of mouth ; *m.a.*, mandibular arch ; *mes.*, mesoblast ; *p.c.*, pericardial cavity ; *ph.*, pharynx. Small crosses indicate the position of mitotic figures. In Figs. 1 and 4 resting nuclei are shown as black spots.

Fig. 1. Transverse section through the ventral part of a young embryo immediately behind the site of the mouth, to show the area of growth in the entoblast preparatory to the formation of the heart endothelium. The dotted circle indicates the position in which the foregut appears in the next section forward.

Fig. 2, *a*, *b*, *c*, *e*. Transverse sections nos. 212, 224, 235, 251 of an embryo in which the first gill slit has just made its appearance. Sections 10 microns thick.

Fig. 3, *a*, *b*, *c*, *d*, *e*. Transverse sections nos. 273, 292, 306, 318, 325 of a later embryo in which the separation of the endothelial cells from the entoblast is nearly completed. Sections 10 microns thick. The sections shown in Fig. 3, *a*, *b*, *c*, *e* are approximately at the same levels as those shown in Fig. 2,*a*,*b*,*c*,*e*, respectively.

Fig. 4. Median sagittal section of the region between the mouth and liver of an embryo of the same age as that shown in Fig. 3. Cell boundaries are shown wherever they can be seen. The heart endothelial cells are evidently continuous with the entoblast behind the mouth, but independent at all other points.

All figures were drawn with Zeiss apochromatic lenses and camera. Figures 1, 2, and 3 were drawn with 16 mm. objective and no. 4 ocular; Fig. 4 with 8 mm. objective and no. 4 ocular, and all have been reduced to one third in reproducing.

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PERIODS OF SUSCEPTIBILITY IN THE DIFFERENTIATION OF UNFERTILIZED EGGS OF AMPHITRITE.

J. W. SCOTT.

While studying the unfertilized egg of *Amphitrite* at the Marine Biological Laboratory, Wood's Holl, Mass., I verified Fischer's[1] result that the eggs could be caused to develop cilia by squirting them from a pipette, by transferring them from one dish to another, or by some other sort of mechanical agitation. I believe however that it is inadmissible to speak of this development as parthenogenesis, meaning the production of a normal embryo from an unfertilized egg. Ciliated, swimming structures result, but their differentiation takes place with only partial or abnormal and usually without any definite segmentation. I will discuss the morphology of these processes in another paper. Lillie[2] has shown clearly a similar differentiation in the *Chætopterus* egg.

In addition to Fischer's results, I found: (1) At least two critical periods in which the egg is highly susceptible to mechanical stimulation, one period thirty to forty-five minutes, the other eighty to one hundred minutes after they are removed from the body and placed in sea-water; (2) slight agitation is more effective in the second period than in the first; rougher handling is better in the first than in the second in which the eggs are more easily broken into fragments. (3) Frequent and moderate squirting after thirty to fifty minutes seems more effective than one hard squirting after the same time.

In my early experiments with certain salt solutions, the results were sometimes discrepant, and there was great variableness in the number of swimming eggs obtained under apparently identical conditions. About this time Fischer's paper came into my hands. He had shown that "parthenogenetic development can be produced by adding a small amount of Ca-salt to sea-water" and by "mechanical agitation." "The unfertilized eggs of *Amphitrite*," he says, "develop to the trochophore stage if, after residence in sea-water from one half to one hour, they be squirted from a small nozzled pipette into another dish of sea-water." "The method is an uncertain one," depending upon "state of ripeness," and a "previous residence in sea-water or in one of the sea-water-salt solution mixtures is essential." He had noticed that some eggs are very sensitive to "mechanical manipulation," but rarely develop when treated "immediately after they are cut out of the body of the animal."

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[1] Fischer, Martin H., *Am. Jour. Phys.*, 1902, III., p. 301.

[2] Lillie, F. R., *Archiv für Entwickelungsmechanik der Organismen*, 1902, XIV., p. 377.

Already convinced that there was a time-factor to be considered, I planned the following series of experiments. In each series a set of eggs, removed from a single female at the same time, was used. Due precautions were taken to prevent fertilization by previously washing the female thoroughly in fresh water. The hands of the operator, the dishes and pipettes used, were carefully sterilized in the same way. For the same reason, sea-water was used which had been raised to a temperature of 60° C., cooled and aërated. After washing in fresh water, the *Amphitrite* was placed in a dish of sterilized sea-water until the eggs were removed. In the following four experiments the eggs were removed from the female at 2.10 P. M. July 30, and were at once transferred very carefully to fresh sterilized sea-water.

Experiment 1.—The object of this experiment was to test the effect of transferring from one dish to another. In order to get a standard amount of agitation, the eggs were allowed to fall, one drop at a time, from the mouth of a pipette held one inch above the water. The different lots of eggs and the time each was transferred are given below:

1	control, transferred	2:10 P. M.
2	transferred	2:27 P. M.
3	"	2:43 P. M.
4	"	2:58 P. M.
5	"	3:13 P. M.
6	"	3:43 P. M.
7	"	4:13 P. M.
8	"	4:43 P. M.

The dishes containing the transferred eggs were left undisturbed until 10 P. M., when eggs were taken from 1, 2, 4, 6, 8 and examined. Care was taken to avoid disturbing those left in the dishes.

The control showed nearly all eggs unchanged; in a few the germinal vesicle had broken down and they were darker (more opaque) in color; a few had started to segment.

Lot 2. The germinal vesicle had broken down in nearly all; a "perivitelline space" found in about 20 per cent., but was rather small in most of this number. Most of the eggs were light (translucent) in color.

Lot 4. An irregular "perivitelline space" in 40–50 per cent. The germinal vesicle was broken down in practically all, the light-colored as well as the dark. There were a few extra-ovates.

Lot 6. The germinal vesicle broken down in nearly all; a "perivitelline space" in 40–50 per cent., irregular in some; a smaller number are blackened.

Lot 8. A prominent "perivitelline space" and contracted protoplasm in 50–60 per cent.; the rest have the germinal vesicle intact.

Amphitrite eggs frequently begin differentiation if left in sea-water entirely undisturbed. This is shown in the above control. The experiment so far disclosed no marked phenomena, and I give the above descriptions to indicate the comparatively uniform development at this time. No evidence of normal segmentation was found at any time in this and the three following experiments.

All the dishes were again examined at 9:30 the next morning, as the advanced stages afford a better means of testing the effects of transference. Below is given the estimated number of ciliated eggs found in 2,000 of each lot. Aside from the swimming eggs, the different lots were in practically the same condition as on the previous evening. No further description is then necessary.

Lot Number.	Time Transferred from Beginning.	Number Ciliated in 2,000 Eggs.
1	0 min.	0
2	17 "	0
3	33 "	10
4	48 "	10
5	63 "	4
6	93 "	60
7	123 "	10
8	153 "	10

Experiment 2.—The object of the experiment was to test the effect of a more violent method of transferring. The eggs were taken up in a pipette and squirted with moderate pressure into the dish of sterilized sea-water from a distance of two or three inches; then water in the dish was taken up three times and squirted at the surface. The control was simply transferred.

An examination of these eggs was made at 10:15 P. M., when their condition was not much different from those in Experiment 1, except that more showed effects of the agitation. The next morning, 10:15 A. M., the following results were obtained:

Lot Number.	Time Transferred from Beginning.	Number Ciliated in 2,000 Eggs.
1 control.	0 min.	0
2	7 "	2
3	23 "	10
4	38 "	40
5	55 "	0
6	70 "	3
7	100 "	20
8	130 "	4
9	160 "	0

Experiment 3.—Eggs were transferred in the following lots and squirted moderately as in Experiment 2. Thereafter they were squirted again moderately at frequent (10–15 min.) intervals, up to 4:40 P. M. Examined at 9 A. M. July 31.

Lot Number.	Time Transferred from Beginning.	Number Ciliated in 2,000 Eggs.
1 control.	0 min.	0
2	32 "	60
3	60 "	0
4	120 "	4

Experiment 4.—The eggs were squirted violently, the water vigorously agitated by squirting with a pipette, and then left undisturbed.

Condition at 8:45 A. M., July 31. Many fragments present.

Lot Number.	Time Transferred from Beginning.	Number Ciliated in 2,000 Eggs.
1 control.	0 min.	0
2	33 "	40
3	60 "	2
4	120 "	0

I have taken the above experiments as typical examples. I have occasionally obtained a much larger per cent. of swimming eggs, frequently a smaller number, and sometimes none. Accepting the number of swimming structures as a fair test of development of this kind, we may make again the following statements :

1. In the differentiation of unfertilized eggs of *Amphitrite*, produced by transference, squirting or other methods of agitation, there are at least two periods in which they are highly susceptible, one thirty to forty-five minutes, the other eighty to one hundred minutes after being put into sea-water.

I have attempted to depict this idea on ordinate paper, shown in the accompanying figures. Abscissas represent time from the beginning of an experiment, ordinates the relative number of swimming eggs in 2,000 of each lot. Fig. 1 gives the results of four experiments produced by different degrees of agitation. Fig. 2 shows all the observations of these four experiments combined in a single line ; where two observations were made at the same time their average is taken (in one case only). The dotted line gives my idea of the curve of susceptibility, as brought about by a moderate degree of shaking.

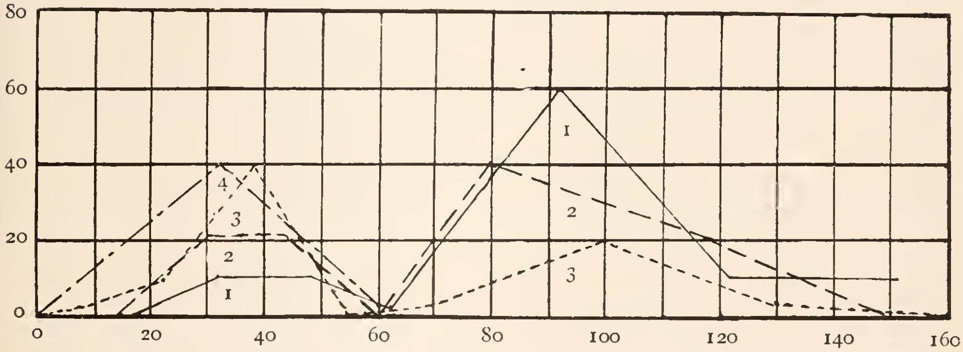

Fig. 1. 1. Gently transferred, experiment 1. 2. Very moderately squirted, experiment 26. 3. Moderately squirted, experiment 2. 4. Violently squirted, experiment 4.

2. By comparing experiments 1, 2 and 3 (Figs. 1, 1, 2, 3) we find slight agitation is more effective in the second period than in the first ; rougher treatment causes more to develop in the first period, but injures some in the second.

3. Frequent and moderate squirting after thirty to forty-five minutes seems more effective than one hard squirting, after the same time. Compare experiments 3 and 4.

A comparison with fertilized eggs is of interest. The normal egg throws off the first polar body in less than thirty minutes after fertilization, and the first cleavage appears about thirty minutes later. According to Loeb's[1] view, the sperm in the case of parthenogenetic eggs acts simply to hasten, or accelerate, processes which are already present in the egg. It hasf requently been noticed that the unfertilized egg of *Amphitrite*, if left undisturbed in sea-water, will often show some phenomena of differentiation. Assuming Loeb's theory as a working hypothesis, we should expect artificial means to be slower than fertilization. This proves to be the case; fertilized eggs develop cilia sooner than those squirted. Sometimes there is not much difference in *Amphitrite*. Presumably, then the two critical periods mentioned correspond to processes in the normal egg that are active about the time for the appearance of the first polar body and the first cleavage; there is the same relative time between them. Further work is needed to prove this. Delage[2] states that the starfish egg is highly susceptible to "artificial fertilization" between the breaking down of the germinal vesicle and the appearance of the first polar body.

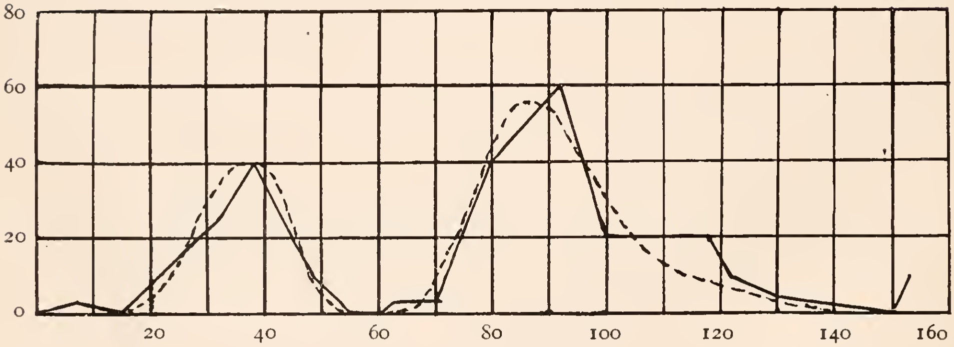

FIG. 2.

However this may be, it is certain that *there are processes of differentiation going on in the unfertilized eggs of Amphitrite which*

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[1] Loeb, J., *Am. Jour. of Phys.*, 1901, Vol. IV., No. IX.

[2] Delage, Y., *Archiv d. Zool. Exper. et. Gen.*, 1901, T. IX., Nos. 2–3.

may be started into activity at definite intervals by mechanical agitation. These processes are, for the most part at least, independent of the processes that cause segmentation. I have noticed, as a rule, that the riper the eggs are the more cleavage is found, but I am convinced that it is never normal beyond the first few segmentations, if at all. It would seem, then, that the sperm introduces the active cause of this process.

It has been shown by Lyon[1] in the fertilized *Arbacia* egg, that there are recurring periods of susceptibility to KNC poisoning, and to lack of oxygen. Each period of susceptibility is followed by a period of resistance. On the other hand, in the unfertilized eggs of *Amphitrite*, there are at certain times unstable conditions, during which a small amount of agitation will set these unstable forces free, and lead to some definite characteristics of more advanced development (*i. e.*, production of cilia, etc.).

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[1] Lyon, E. P., *Am. Jour. of Phys.*, 1902, Vol. VII., No. 1.

FURTHER STUDIES ON THE EFFECT OF VARIATIONS IN THE TEMPERATURE ON ANIMAL TISSUES.

ARTHUR W. GREELEY.

This paper contains an account of a series of experiments which are the outcome of others of a similar nature described in two earlier papers by the same author.[1] This previous work has called attention to the fact, noted by other observers, that the fluidity of the protoplasm of any of the Protozoa studied, varies directly with the temperature up to a certain critical point (28°–35° C.), above which the protoplasm suddenly goes into heat rigor, or coagulates. My own work has shown that as the temperature is lowered below the normal, a similar coagulation sets in which causes the cell to lose water.[2] This loss of water and coagulation is accompanied by a gradual cessation of the vital activities of the cell, and brings about certain very definite morphological changes that result in the formation of resting cells, which consist only of an undifferentiated mass of protoplasm. In the case of *Monas*, these changes were carried further by exposing the cells to a still greater reduction in the temperature, and the resting cells were finally broken up into many small spores, each of which reproduced the motile organism when returned to the normal temperature. As the temperature is raised above the normal, the protoplasm takes up water and all its vital activities are accelerated, until coagulation suddenly ensues at the critical point.

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[1] Greeley, *American Journal of Physiology*, 1901, VI., p. 122. BIOLOGICAL BULLETIN, 1902, III., p. 165.

[2] This fact that lowering the temperature to 1° to 5° C. and raising the temperature above the critical point has the same effect upon protoplasm (*i. e.*, coagulation and loss of water), has received an interesting verification in the recent work of Fischer on Lepidoptera (*All. Zeit. für Entomologie*, October 15, 1901). In experiments on the artificial production of seasonal varieties of *Vanessa anteopa* by exposing the larvæ to different degrees of temperature, Fischer discovered that precisely the same variations in the adult forms are produced by lowering the temperature to 1° C. or raising it to 40° C., while modifications in the temperature within those limits gave strikingly different results.

In order to determine whether similar structural changes, as have already been described in the cases of *Stentor* and *Monas*, could be produced on other forms as well, the low temperature experiments have been continued on many other Protozoa, both Infusoria and Rhizopoda, and in all of them changes identical with those described above have been obtained. *Monas* is the only form in which it has been found possible to control the formation of spores, but in all the others resting cells were formed at the low temperature, which reverted to the motile condition when restored to the normal temperature.

I. The Reversal of Vital Phenomena by a Reduction of the Temperature.

The results of these low-temperature experiments on the Protozoa suggested an interesting comparison to the experiment of Loeb's,[1] in which the tentacles and polyps of a Campanularian Hydroid were reduced to the undifferentiated protoplasm of the stolon by bringing them in contact with some foreign substance. It appeared that for the Protozoa a lowering of the temperature as well as a contact stimulus brings about just such a reversal of the vital phenomena until the undifferentiated resting cell is formed, while a small increase in temperature accelerates the metabolic processes. To see if a lowering of the temperature brought about similar changes in the more complex multicellular animals a series of experiments was begun on the fresh-water Hydra.

It was at once observed that whenever a Hydra is exposed to a temperature of 4° to 6° C., the tentacles gradually become shorter and thicker, and are finally completely absorbed into the body. As the absorption goes on, the ectoderm and entoderm cells of the tentacles lose their individuality and form an undifferentiated mass of protoplasm, which is slowly taken into the body of the Hydra (see Fig. 4). The tentacleless body of the Hydra becomes slowly resolved into a dense spherical mass of coagulated protoplasm, in which no distinction between the individual cells can be made out, and remains in this condition as long as it is kept at a low temperature (see Fig. 3), but quickly forms tentacles and a double layer of cells again when it is returned to the temperature of the room. Thus a lowering of the temperature seems to produce essentially the same effect on Hydra as the contact stimulus on the Campanularian Hydroid in Loeb's experiment. Likewise the structural changes appear to be identical with those produced by the low temperature upon the Protozoa.

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[1] Loeb: *American Journal of Physiology*, 1900. *IV.*, p. 178.

Hydra react to variations in the temperature in another way which is interesting when compared to the reactions of Protozoa under the same conditions. It has been a fact of common observation that the rate of cell division varies directly with the temperature for all temperatures below the critical point. In my experiments on *Stentor* I showed that a lowering of the temperature not only inhibits cell division but brings about the reverse process. If a *Stentor* in the process of division be placed at a

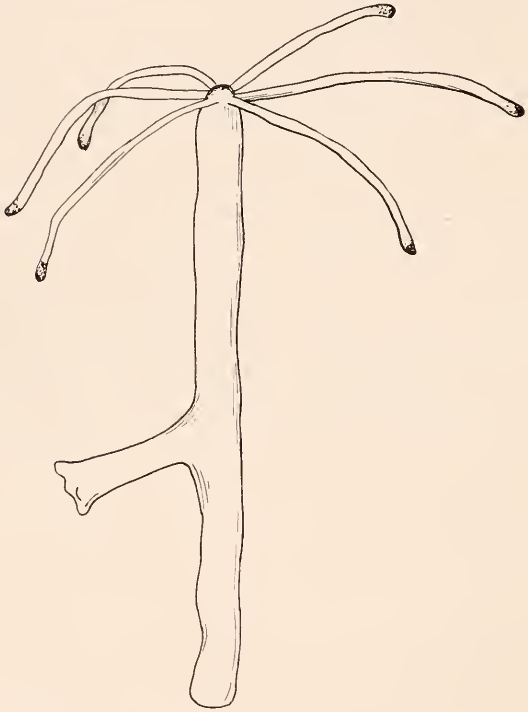

FIG. I. A budding Hydra after an exposure of twenty-four hours to a tempera ture of 6°C. The body is slightly shortened and thickened, and the absorption of the ectoderm and entoderm cells has begun in the tips of the tentacles.

temperature of 4°C. a fusion of the partially divided halves takes place. Among Hydra the formation of buds, which finally become distinct individuals, may be considered analogous to the process of cell division among Protozoa. It was found that if a Hydra in the earlier stages of the process of budding be placed at a temperature of 4°C., not only does the growth of the bud stop instantly, but an absorption of the bud into the body of the parent commences, and continues until all traces of the bud have disappeared. (See Figs. 1 and 2.) In order to demonstrate this absorption of the bud, great care is needed in lowering the temperature. If the temperature is quickly reduced to 1°C. the Hydra go to pieces, but if the temperature be maintained at from 4° to 6°C., and is not suddenly varied in either direction, the process of absorption can be easily seen. Six or seven days are required for the complete disappearance of the bud. These two experiments seem to show that among the Cœlenterates as well

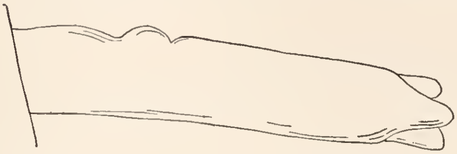

FIG. 2. The same Hydra as in Fig. 1, after an exposure of six days to a temperature of 6°C. The absorption of the tentacles and bud is nearly complete.

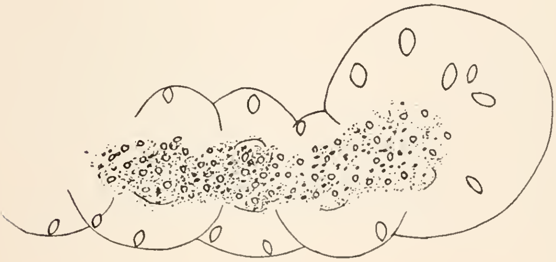

FIG. 4.

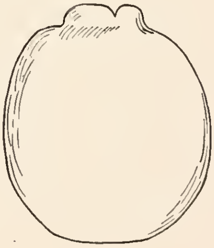

FIG. 3.

FIG. 3. The final resting stage of Hydra, formed after an exposure of four to seven days to a temperature of 6°C. The body consists of an undifferentiated mass of protoplasm.

FIG. 4. The tip of a tentacle of a Hydra that has been exposed to a temperature of 6°C. for twenty-four hours, showing the dissolution of the octoderm and endoderm cells.

as the Protozoa, a lowering of the temperature brings about a reversal of the vital phenomena and the formation of simple resting stages.

II. The Effect of Variations in the Temperature Upon Developmental Processes.

It has been frequently observed that eggs, spores, cysts, or other resting stages of the motile organism which are formed to tide over some unfavorable conditions in the life-history of the animal, will not develop into the motile form unless they are exposed to the very conditions that brought about their formation, and normally intervene before development commences. Thus Braem[1] found that the statoblasts of Bryozoa, and the winter eggs of *Apus* would develop only after they had been exposed to a certain degree of low temperature. In this case the physical change produced in the protoplasm of the egg or statoblast by the low temperature seems to be necessary before the developmental processes can originate.

Dr. Loeb suggested to me the possibility that the same thing might be true for the development and metamorphosis of the chrysalids of a common moth, *Cecropia*, that are formed in the Autumn, but do not complete the metamorphosis until the following Spring. To test this hypothesis and see if other means besides that of low temperature would suffice to start development, the following series of experiments was performed:

On October 15, 1901, before they had been exposed to any frosts, a large number of cocoons were brought into the laboratory. Many of these chrysalids were found to be parasitized by an ichneumon fly, and only a small number were available for the experiments. The cocoons were kept in the laboratory at a temperature of 20° C. until November 27. They were then divided into two lots. One lot was kept constantly at a temperature of 20° C., as a control series, and the other was placed outdoors for six days, at a time when the temperature fell below 0° C. each night. At the end of the six days, these cocoons were brought back into the laboratory, and kept with the others at a temperature of 20° C. On January 27, the chrysalids that had been exposed to the low temperature began to produce moths, and all of them had completed the metamorphosis by February 3. None of the chrysalids that had been kept at a temperature of 20° C. showed any signs of development. Several of the cocoons were opened, but the chrysalids were in the same condition, as far as could be seen, as when they were collected.

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[1] Braem, *Jahrber. Schles. Ges. f. nat. Cult.* (Zool. Bot. Sec.), 1895, p. 2.

This result indicated that a lowering of the temperature at least accelerates the metamorphosis of the chrysalids. To determine whether the effect of the low temperature on the larva consisted in an extraction of water from the protoplasms, as was the case in the low temperature experiments on the Protozoa, the experiment was now varied as follows: The cocoons, that had been kept constantly at a temperature of 20° C., were now, on February 3, divided into three lots. One lot was retained at the room temperature, 20°C., another lot was exposed outdoors to a temperature of about — 10° C. for seven days, and the third lot of four cocoons was placed in a desiccator over sulphuric acid for two days. These four cocoons, while in the desiccator, lost water as is shown by the following record of weights:

	Weight when Placed in Desiccator, Feb. 3.	Weight when Removed from Desiccator, Feb. 5.
1.	17.0555 g.	17.031 g.
2.	15.184 g.	15.173 g.
3.	16.630 g.	16.603 g.
4.	15.115 g.	15.095 g.

These four cocoons produced moths on March 4, 10, 13 and 14. On March 24, moths emerged from the cocoons of the second lot that had been exposed to the low temperature, but on March 26 the cocoons of the control series that had been kept continuously at the room temperature produced moths also, showing that this last exposure to a low temperature was too late to have any effect. The desiccation hastened the development by about two weeks. We see from this experiment that the original exposure to a low temperature in November, soon after the cocoons were first brought into the laboratory, hastened the development by two months, and that the desiccation within two months before all the cocoons produced moths sufficed to accelerate the development materially. These experiments are far from satisfactory because of lack of material, but they furnish testimony to the conclusion already reached by Braem and others, that in resting stages of this sort, development can commence only after some physical change has occurred in the protoplasm through the action of a low temperature or other changes in the external conditions. These experiments further seem to show that the changes produced in the protoplasm by lowering the temperature are identical with those produced by an extraction of water, as has already been indicated in the experiments on Protozoa.

It is interesting to note that the same forms of stimuli (*i. e.*, a lowering of the temperature and an extraction of water), which hasten the development of the moth, also produce artificial parthenogenesis of the starfish egg. This fact lends weight to the idea, expressed by Loeb, that artificial parthenogenesis consists merely in the acceleration of developmental processes already present in the egg.

III. Effect of Temperature on the Absorption of Water by Muscle.

If the conclusion drawn from these earlier experiments, that a reduction of the temperature produces changes in the protoplasm that cause it to lose water is true, then variations in the temperature ought to have a decided effect on the absorption of water by muscle or other animal tissue. The experiments of Loeb[1] and Webster[2] on the gastrocnemius of the frog have demonstrated that this muscle always behaves in a very constant way, as far as can be determined by its change in weight, toward each salt solution in which it is immersed. In some salt solutions the muscle always absorbs a definite amount of water at the normal temperature, in others of the same osmotic pressure it always loses a definite amount. The only variation in this behavior of the muscle toward salt solutions occurs with the change of seasons, the muscle of winter frogs differing widely from summer frogs in this respect. This fact had been the only indication that temperature in any way affected the absorption of water by the frog's muscle, and the influence of the temperature alone was not clear in this case. In order to ascertain the influence of temperature upon this process and to obtain, if possible, some quantitative estimate of its action, I started a series of experiments to test the absorption of water by frog's muscle in the same solution at different temperatures.

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[1] Loeb, *Archiv. f. d. ges. Physiol.*, 1899, LXXV., p. 303.

[2] Webster, Univ. of Chicago Decennial Publications, 1902, X., p. 105.

All the salt solutions were used at dilutions isotonic with *m*/8 NaCl which is supposed to represent as nearly as possible the average osmotic pressure of the muscle. When tested at the normal temperature (20°C.), the solutions of all the salts experimented with, fall into one of three classes: first, those solutions which cause the muscle to absorb water as is shown by its increase in weight, for example, the univalent salts, KCl and NH_4Cl, and salts with a bivalent anion and two univalent kations as Na_2SO_4; second, those solutions which cause the muscle to lose water as shown by its decrease in weight, for example, salts with a bivalent kation and two univalent anions like $CaCl_2$ or or $SrCl_2$; third, those solutions which leave the water content of the muscle unaltered. LiCl is the best example of this third class. *m*/8 NaCl usually falls in this group, although in my experiments, I found that *m*/8 NaCl caused a slight increase in weight, and that *m*/6 NaCl was more nearly isotonic with the muscle.

The method used in the experiments was the same one that has been elaborated so successfully by Webster.[1] A large amount of each solution was made up isotonic with *m*/8 NaCl, and was then divided among dishes which were kept constantly at the following temperatures: 1°C., 20°C., 25°C., 27°C., 29°C., 31°C., 36°C., 38°C., 45°C. and 55°C. The gastrocnemius muscle of the frog was used in the experiment. The muscles were carefully weighed and then distributed among the dishes containing the solution to be tested at the temperatures named above. The muscles were weighed after remaining in the solutions for three hours, and again after twenty-four hours, and the gain or loss in the water content calculated in percentages of the original weight of the muscle.

The results of the experiments are given in Table I., in which are given curves showing the effect of temperature on the absorption or extraction of water after the twenty-four-hour exposure to each of the solutions. The curves for the three-hour exposure to the solutions are not given, because the twenty-four-hour

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[1] Webster, *loc. cit.*

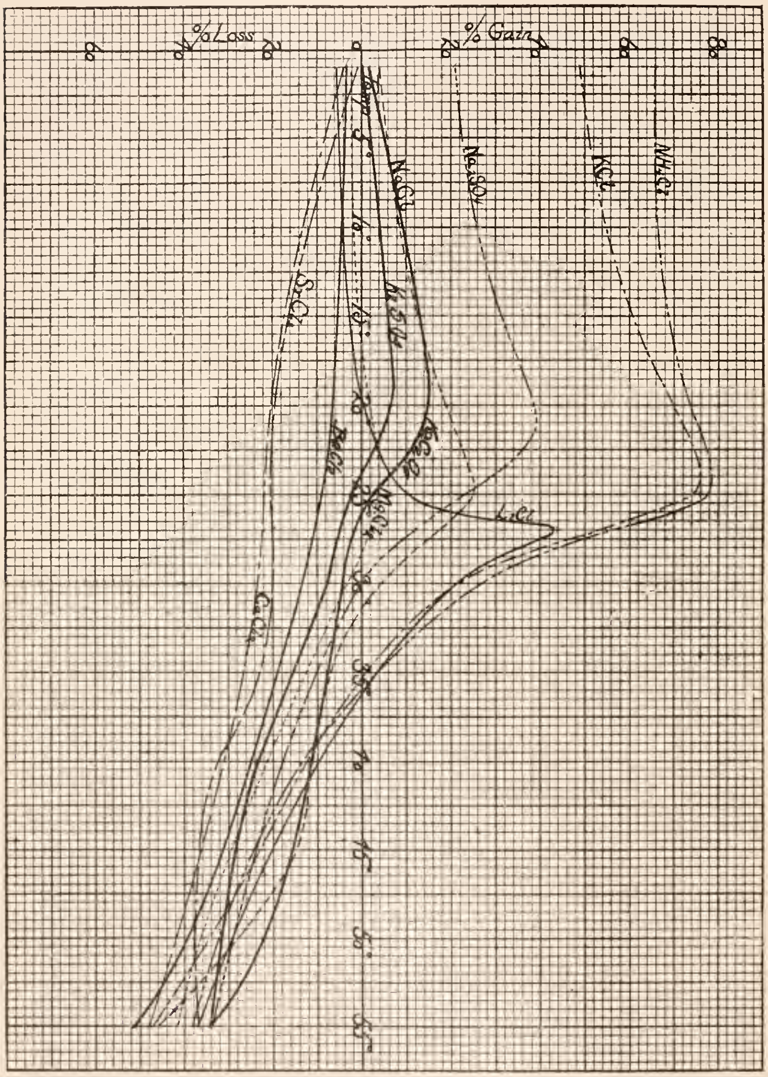

TABLE I. Curves showing the effect of temperature on the absorption of water by the gastroc-nemius muscle of the frog in various solutions isotonic with $m/8$ NaCl. The period of exposure to the solutions was twenty-four hours.

curves are entirely typical of the results obtained in both cases.

In considering the temperature effects we may classify them as regards their bearing on the absorption phenomena in the three classes of solutions mentioned above.

First, of those salts whose solutions, isotonic with *m*/8 NaCl, cause an absorption of water at the normal temperature (20°C.), the following were used: NaCl, KCl, NH_4Cl, Na_2SO_4, K_2SO_4 and $K_2C_2O_4$. In all of them, as is shown by the curves, the absorption of water varies directly with the temperature up to a certain critical point, in the neighborhood of 25°C., at which a sudden loss of water commences which increases rapidly with a further elevation of temperature. The form of the curve is the same for all the solutions, regardless of whether the initial absorption is great or small, and at about 50°C. the loss of water becomes practically the same in all the solutions. The form of these curves is strikingly like that showing the direct effect of temperature upon the amount of water in protoplasm, independently of the specific action of any salt solution, as is shown by the curve for *m*/8 NaCl which approximates as nearly as possible the fluid which normally bathes the muscle during life, as far as its chemical composition is concerned. For these reasons it seems probable that the effect of temperature upon the absorption of water in these solutions is due to the physical changes induced by the variations in the temperature in the protoplasm of the muscle. The rise in temperature may also accelerate the specific chemical action of the solution upon the muscle proteids, but in any event this only increases the result produced by the temperature alone. The amount of water in the protoplasm of a Protozoan varies directly with the temperature up to the critical point which marks the beginning of heat rigor, and it is interesting to find that the same thing occurs in muscle when immersed in solutions which are isotonic with its own substance. Above the critical point the heat rigor causes the same loss of water in all the solutions regardless of their chemical composition.

The same temperature effects are still better shown in curves of the absorption in solutions of the second class, *i. e.*, those which cause neither a gain or loss of water in the muscle at the normal temperature. Of the solutions of these salts, three were used: LiCl, $MgCl_2$ and $m/6$ NaCl. Usually $MgCl_2$ has been described to act like $CaCl_2$, $BaCl_2$ and $SrCl_2$, in whose class it would naturally fall, in causing a loss of water. But although I tested its action many times, in all my experiments it had practically no effect on the weight of the muscle at the room temperature. It will be seen by examining the curves for LiCl and $MgCl_2$, that at a temperature of 1° C., the muscle loses a small amount of water. This loss of water decreases steadily as the temperature is raised until just above 20° C., an absorption of water commences, which increases until the critical point is reached. Above this point the muscles lose water very rapidly just as in the other solutions. Thus in these solutions, which appear to have no effect on the muscle at the normal temperature, there is a loss of water at low temperatures, a gain at temperatures between the normal and the critical point, and a very rapid loss above the critical point, which is exactly the effect that changes in temperature have been shown to have on the protoplasm of the cells of Protozoa, when only the physical condition of the protoplasm is modified by the variations in the temperature. $m/8$ NaCl also should have no effect on the amount of water in muscle at the room temperature, but in many cases, especially with the muscles of winter frogs, this solution appears to be hypotonic to the muscle substance. As Webster has shown, the osmotic pressure of the muscle varies with the season of the year, being higher during the winter, which is the condition we should expect from the observed effect of low temperatures on protoplasm. In my experiments (with winter frogs), $m/8$ NaCl invariably caused a slight gain in weight, but $m/6$ NaCl was found to be isotonic with the muscle, and the curve for this solution corresponds exactly with that for LiCl. Thus in all these solutions which appear to have no chemical effect on the muscle, as far as can be determined by the changes in weight, the amount of water in the muscle varies directly with the temperature up to the critical point, and inversely with the temperature above that point, and it is reasonable to suppose that the same thing occurs in the muscle in its normal surroundings within the body.

The curves for $CaCl_2$, $BaCl_2$ and $SrCl_2$ are very different from those of the first two classes of salts. Solutions of these salts, isotonic with *m*/8 NaCl, cause a loss in weight of about 20 per cent. at a temperature of 20° C. It will be seen from the curves that in each of these solutions the loss of water is very slight at a temperature of 1° C., and that the decrease in weight increases steadily as the temperature is raised. But at a temperature of 50° the physical changes in the protoplasm overbalance the specific action of any solution and the muscles lose practically the same amount of water in all solutions. It appears that in the case of these solutions we are dealing with specific ion effects, as Loeb[1] has already suggested, and that the curve may be interpreted as follows: The speed of any chemical combination varies directly with the temperature. At a temperature of 1°C. the reaction between Ca, Ba or Sr and the muscle proteids is so greatly slowed that the solution has no effect on the muscle, and the small loss of water is due entirely to the physical changes in the muscle produced by the low temperature, as in LiCl, *m*/6 NaCl, and the other solutions which have no specific action on the muscle substance. As the temperature is increased, the reaction between the muscle proteids and the ions is accelerated, and this chemical action of the Ca, Ba or Sr ion overcomes the effect of the physical changes produced by the temperature, and the loss of water steadily increases because these ion proteid compounds, like Ca-soaps, hold very little water. It is worthy of notice, however, that at about 25° C. there is a break in the continuity of the curves, corresponding with the rapid absorption of water in the other solutions, which indicates a change in the physical condition of the protoplasm that neutralizes temporarily the specific ion effects.

In distilled water the amount of absorption by the muscle is decreased by lowering the temperature, as is shown by the following result for a one-hour exposure to distilled water: Percentage of absorption

at 2° C.	40.7
at 30° C.	53.1

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[1] Loeb, *loc. cit.*

SUMMARY.

1. In Hydra as well as Protozoa, a lowering of the temperature brings about certain definite structural changes that result in the formation of an undifferentiated resting stage.

2. The inhibition of cell division and reversal of vital phenomena by a reduction of the temperature is shown in Hydra by the fact that at a temperature of 6° C., the growth of a new bud ceases, and the partially formed bud is gradually absorbed into the body of the parent animal.

3. A lowering of the temperature and an extraction of water both bring about the same physical changes in the protoplasm which serve to accelerate the development and metamorphosis of the chrysalids of *Cecropia*.

4. The absorption of water by the gastrocnemius muscle of the frog in those salt solutions which, when used at dilutions isotonic with its own substance, either have no chemical effect on the muscle at the room temperature, or cause an increase in weight, varies directly with the temperature, until the critical point is reached at which the muscle proteids begin to coagulate. In solutions of the same osmotic pressure, which cause the muscle to lose water at the room temperature, this loss of water varies directly with the temperature. Above the critical point of temperature the muscles lose practically the same amount of water in all solutions, regardless of their initial effect on the muscle.

ZOOLOGICAL LABORATORY,
WASHINGTON UNIVERSITY, March 18, 1903.

THE EMBRYONIC DEVELOPMENT OF THE OVARY AND TESTIS OF THE MAMMALIA. (PRELIMINARY ACCOUNT.)

BENNET M. ALLEN.

The following is a preliminary account of work begun in the spring of 1900, upon the subject of the development of the ovary and testis of the mammalia. The rabbit and pig have served as the subjects for this work. The results will here be very briefly set forth, a more detailed account being reserved for a later paper. The material studied includes various stages in the development of the ovary and testis of the rabbit, from the thirteen-day embryo to and including adult stages. The pig material includes only embryonic stages, but is more complete for the period covered than is the rabbit material.

The work was carried on with the aim of solving the following problems: (1) the origin and development of the seminiferous tubules and their homologues in the ovary; (2) the origin, development, and homologies of the rete tubules, also their relations to the Malpighian corpuscles of the mesonephros on the one hand, and to the seminiferous tubules of the testis and medullary cords of the ovary on the other; (3) the origin, development and homologies of the connective tissue elements and interstitial cells of the ovary and testis.

Incidental to the solution of these problems, the work has involved to a greater or less extent, a consideration of the following allied problems: (1) the development of sex cells; (2) the morphological phases of sex differentiation; (3) cell degeneration in the sex gland and rete region, (4) the degeneration of the mesonephros, and the development of the Wolffian and Müllerian ducts.

The results of work in so large a field can be, only to a limited extent, new. Certain of the following results are confirmatory of the work of other authors, to whose results I shall refer in a later paper. The very contradictory opinions met with in the literature on the subject call for confirmatory evidence upon these problems.

The earliest rudiment of the sex gland is situated in the genital ridge, which consists of a zone of thickened peritoneum, running the entire length of the mesonephros, parallel and close to the mesentery which unites the latter to the body wall. The rete is formed from the anterior part of the genital ridge and extends, in the pig, from about the region of the sixth glomerulus to a point about opposite the twentieth glomerulus, as shown in a number of series. However, these limits are variable. In the rabbit the limits are more constant, but still variable, the rete extending approximately from the sixth to the twelfth glomerulus. The anterior end of the sex gland rudiment slightly overlaps the posterior end of the rete region. Posterior to the sex gland is a section of the genital ridge that does not develop beyond the very early formation of a region of thickened peritoneum. In each of these three zones of the genital ridge are found scattered cells with distinct cell walls, clear cytoplasm, large round nucleus, centrosphere and centrosome — the so-called primitive sex cells.

In its early stages of development the genital ridge consists of a thickened layer of peritoneum overlying a loose mesenchyma; the cells of the latter are to all appearances identical with those of the peritoneum, from which they undoubtedly originate. This resemblance applies both to the character of their nuclei and to their lack of definite cell boundaries. The peritoneal layer and underlying mesenchyma are separated from one another by the basement membrane of the former. This is formed by the interlacing of protoplasmic fibrils given off by the cells of both layers. In later stages, one finds a progressive crowding of the peritoneal nuclei. In the rete and sex gland regions this results in the formation of tubular peritoneal invaginations, which are limited from the surrounding mesenchyma by the persistent membrana propria. In the sex gland region these tubular cords of cells may be known as the sex-cords. At this indifferent stage they are closely massed together side by side, and the very narrow interspaces between them contain scattered mesenchyme cells, which from now on, may be considered under the general term of stroma. This is used to designate the loose connective tissue of both ovary and testis. Invaginations, in all respects similar to these sex-cords, arise from the peritoneum of the rete region. They lie further apart than do the sex-cords, and penetrate more deeply into the mesenchyme. Both rete-cords and sex-cords are at this stage devoid of a lumen. In cross-section they show a limiting membrana propria, within which is a single layer of cells arranged with their bases attached to the membrana propria and their apices meeting at a common point in the center — the rudiment of the lumen.

The mesentery of the sex gland is formed by the proliferation of connective tissue cells from the peritoneum, in areas of restricted width immediately ventral and dorsal to the sex gland. The ventral area is by far the more important of the two sources. The albuginea is in large part formed from the cells comprising the proximal parts of the sex-cords. They are formed at the time when the sex gland has begun to assume definite shape. At this time the rapidly dividing cells of the attached ends of the sex-cords have become differentiated from those of the more distal portions in that they elongate, become smaller, and acquire the property of staining more deeply. They then break away from the peritoneum on the one hand, and from the sex-cords on the other. They may still for some time be found attached to portions of the membrana propria that ensheathed them. They become mingled with certain exactly similar intertubular mesenchymal elements, to form the albuginea, which is essentially one with the remaining connective tissue or stroma of the sex gland. Sexual differentiation is first manifested by the cessation of growth of the sex-cords of the ovary. We can then distinguish them as medullary-cords. The peritoneum of the ovary begins to increase in thickness, and eventually forms the cortex of the adult ovary in a manner to be briefly indicated below. The albuginea of the ovary forms a broader, looser, and more irregular layer than does that of the testis. In the testis the peritoneum ceases to grow, in large measure at least, its cells becoming flattened, and in later life practically disappearing. The rete cords grow backwards from their points of origin, and enter the anterior part of the sex gland. They branch and anastomose throughout their course, sending branches to the Malpighian corpuscles on the one hand, and on the other to the seminiferous tubules of the testis. The branches passing to the Malpighian corpuscles meet evaginations from the capsules of Bowman, with which they fuse. Such evaginations are irregular in number, as many as three having been counted upon the same glomerulus. Some glomeruli may send out none at all. The tubuli recti connecting the rete-cords with the seminiferous tubules are likewise irregular in number, being apparently called forth wherever needed.

Seminiferous tubules, medullary-cords, and rete-cords are homologous structures. Not only are they of similar origin, but their component cells show similarities. Two kinds of cells are found in all three structures: (1) primitive sex cells, which have already been described; (2) cells more or less variable but agreeing with one another in not having clearly marked cell limits, and also in the absence of centrosphere and centrosome. The cells of this second class form the germinative cells of the seminiferous tubules, the follicular cells of the medullary-cords, and the rete cells proper of the rete-tubules.

Returning to the subject of the ovary, the peritoneum at the time of separation of the medullary-cords, contains no differentiated sex cells. Such may exist, but they are at all events indistinguishable from the remaining peritoneal cells. The peritoneum or germinal epithelium, as it may now be termed, next begins to give rise to the cords of Pflüger, which branch and anastomose in a similar manner to the medullary-cords. Some of these cords of Pflüger may contain a well-defined lumen, in the case of advanced embryos of the pig (15 cm. length). In these later stages the inner ends of the cords are broken up to form follicles. Follicles are likewise formed in medullary-cords. These however, have never more than one layer of follicular cells in the forms studied.

The rete-cords come in contact with the medullary-cords, and are then scarcely distinguishable from the latter in the case of the rabbit. They contain no sex cells in later stages of the ovary of that animal, although such are present in the rete tissue when it is first laid down. By this criterion alone can one, in a very general way, distinguish between medullary-cords and rete-tissue lying within the rabbit's ovary. In the pig, on the other hand, the rete-tissue shows some very interesting characteristics. The portions of the rete-cords lying within the ovary undergo similar differentiation to the medullary-cords and the cords of Pflüger forming the cortex. The rete-tissue within the ovary of the 18 cm. pig embryo is found to contain young follicles, each with a single layer of follicular cells; the enlarged oöcyte in the center having passed through the synapsis condition, characteristic of one stage in the development of the young oöcytes. All such follicles subsequently degenerate. In the testis the intra-glandular portions of the rete-tubules are similar to the seminiferous-tubules, but differ from them in their much smaller diameter and in the earlier acquisition of a lumen. They contain the sex cells characteristic of the seminiferous-tubules. These are at first present in the extra-glandular region of both ovary and testis, but disappear more or less completely in later stages. No attempt was made to study out the fate of the sex cells of the rete-tubules of the pig testis. They are still present in the 25 cm. pig embryo. In the rabbit they are found in the rete of the testis twenty-four days after birth, but are not to be found in that of a rabbit killed 140 days after birth. The rete-tubules are so completely united by anastomosis that their connected lumen forms a large irregular cavity divided here and there by irregular partitions formed by the walls of the several rete-tubules.

The connective tissue elements of ovary and testis are derived from the peritoneum. In early stages they are not distinguishable from the cells that make up the sex-cords, except that the latter are marked off from the stroma by their membrana propria. As before stated, the albuginea is largely formed by actual transformation of the basal part of the sex cords into connective tissue elements.

The interstitial cells are characterized by a large nucleus, distinct cell boundaries, a centrosphere and centrosome, and very granular cytoplasm. They first appear in the stroma of both testis and ovary of the pig of 2.5 cm. length. They are far more numerous in the testis than in the ovary. Their appearance is coincident with that of a large number of fatty globules in the peritoneum and sex cords. In the testis they persist for a long time. In the ovary, however, the few cells appearing at this stage speedily disappear. In both organs they divide by mitosis.

This process soon ceases in the ovary, while in the *testis*, on the other hand, division figures are found in the interstitial cells at a stage as late as the 7.5 cm. embryo. In the testis of the 15 cm. embryo, they have begun to degenerate. This process manifests itself in a shrinkage of the cytoplasm. Interstitial cells first form in the testis of the rabbit embryo of a stage between seventeen and twenty-one days. They are found to be still dividing by mitosis eight days after birth. They are very rare, however, in the stage of twenty-four days after birth.

No interstitial cells were found in the *ovary* of the embryo rabbit, they being first met with in females killed forty-five days after birth. Here they are scarce, but unmistakable. Considerable light is thrown upon their origin by a study of the eighty-five-day rabbit. In the ovary of this stage they are very common, their origin from the cells of the theca interna of atretic follicles being clearly shown. This, taken in connection with the additional fact that they make their appearance in the 2.5 cm. pig embryo coincident with the fatty degeneration of the germinative cells of the seminiferous tubules and their ovarian homologues, together with that of many cells of the germinal epithelium, would lead us to conclude that cell degeneration offers the stimulus or condition that brings about the formation of the interstitial cells.

Interstitial cells do not develop from unmodified connective tissue cells, such as those comprising the theca externa and the general ovarian stroma. Such stroma cells must be transformed into cells of the theca interna by the direct or indirect influence of the growing follicles, before they are again susceptible to the influences exerted by the process of cell degeneration. Atresia of small follicles that are not surrounded by a theca-interna does not bring about the formation of interstitial cells. Many such small follicles are found to degenerate early and late in the history of the ovary.

No evidence has been found favoring the theory of the early segregation of the sex cells, but I am not prepared to say that my work has in any way tended to disprove such a theory. Sex cells appear in the very youngest stages studied (pig embryo, 0.6 cm. length and rabbit embryo of 13 days' age). They are most common in the region where the sex-gland will eventually form, occurring both in the peritoneum and among scattered subperitoneal cells of mesenchymal nature. They are prominent in the sex-cords of a later stage. In the 1.8 cm. pig embryo, immediately after the separation of the sex-cords from the peritoneum, the latter is found to contain no sex-cells distinguishable as such. If the sex-gland be an ovary, they soon (2.5 cm. pig embryo) make their appearance in the peritoneum, and especially in the cords of Pflüger growing inward from it. These cords of Pflüger increase by growth at their bases, *i. e.*, their points of connection with the peritoneum. Hence there is a continual development of peritoneal cells to form the primitive ova distinguishable as such. The case of the seminiferous tubules is not so clear. Well-developed sex cells are found in them from the start. On the other hand, all stages of transition are found to link the germinative cells with the sex cells. These transitional cells are found in the testis of the pig at as late a stage as the 13 cm. embryo.

Whether the sex-cells that appear in the very early stages of embryonic development ever produce functional sex products in the testis, is a question that cannot easily be solved in this form. Certain it is, however, that true sex products do form in both ovary and testis from apparently undifferentiated cells of peritoneal origin, and that those which are functional in the ovary form *exclusively* from this source. The sex-glands and rete tissue are the seat of extensive processes of cell degeneration. I shall not here enter upon a discussion of the different forms which this process assumes, but shall defer treatment of these considerations to the more complete account.

This piece of research has brought up many interesting facts, bearing upon questions touching upon the action of trophic stimuli in embryonic development. Perhaps the most striking example of this is the formation of follicles in that portion of the rete tissue lying within the ovary. The extra-ovarian part, or that remaining in the mesonephros, does not contain follicles, although it is of precisely the same origin as the intra-ovarian portion. The influence of the ovary reaches out a short distance into the mesonephros, as can be seen by the presence there of a few sex cells, which are more numerous in the regions nearest to the ovary. There is a definite interaction between the capsules of Bowman and the rete-cords lying nearest to them. Each sends forth a process to meet the other. In the testis the rete cords send out processes (tubuli recti) to meet the seminiferous tubules at their inner ends. Each tubule receives its rete branch. A large number of tubuli recti can arise, from a single rete-tubule at different points in its course. The connection between cell degeneration and the formation of interstitial cells has already been discussed. The uriniferous tubules of those glomeruli with which the rete-cords come in contact persist as the rete efferentia of the male, while the remaining ones disappear wholly, or in large part at least. A few rudiments of these rete efferentia tubules persist in contact with the rete ovarii. Such rudiments are very rare in the pig embryo of 25 cm. length.

Hull Zoological Laboratory,
University of Chicago, April 20, 1903.

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BIOLOGICAL BULLETIN.

BUNODERA CORNUTA SP. NOV.: A NEW PARASITE FROM THE CRAYFISH AND CERTAIN FISHES OF LAKE CHAUTAUQUA, N. Y.

HENRY LESLIE OSBORN.

A trematode is met frequently at Chautauqua, New York, which though already known seems never to have been critically studied and described. While generically identical with *B. nodulosa* Zeder, of Europe, it cannot be referred to the same species. A form mentioned by Kellicott, '83, and Wright, '84, and Linton, '92, may be identical with it. I have not yet had access to the articles of the first two writers, but Linton, '92, regards the form they mention as identical with the one which he describes from cysts from the ovary of crayfish from Alma, Michigan, which, while it is much like *B. nodulosa* of Europe, he regards as distinct, on account of the two lateral papillary appendages projecting from the oral sucker and of triangular shape. Ward, '94, reports at Ann Arbor, Mich., the form mentioned by Kellicott, Wright and Linton, and considers it probably identical with *B. nodulosa* of Europe. I am at present inclined to think that of these four cases at least that of Linton is identical with the Chautauqua form, and that the others may be. My knowledge of *B. nodulosa* is almost entirely drawn from the account of it in Looss' ('94) admirable monograph of the fish and frog distomes, as I have not had access to specimens of that form. A related trematode is described and figured by Linton, '98, from the intestine of the lake sturgeon, and referred to *B. auriculata* of Wedl, '57. A single specimen of the material on which Linton's account was based has been loaned me by the U. S. National Museum through the kindness of Dr. C. W. Stiles, and from such examination of it as I could make without injuring it I was able to see that externally it is essentially like *B.*

cornuta, excepting as regards the oral papillæ. On this point and in the figures of Linton, there is a divergence from either *B. nodulosa*, for the ventral papillæ are transverse and in the form of a horn, and from the Chautauqua form for the four anterior papillæ characteristic of both *nodulosa* and *cornuta* are wholly wanting. If the absence of these papillæ is a constant character, as at present it must be assumed to be, we then must accept three species for this genus. The coarser features of the organization of the Chautauqua form is described in the following pages. An account of some points in the minuter structure is reserved for a later article. It will be necessary and convenient at least till more is known of *B. auriculata* to adopt a name for the Chautauqua species, and I propose for it the name *B. cornuta*.

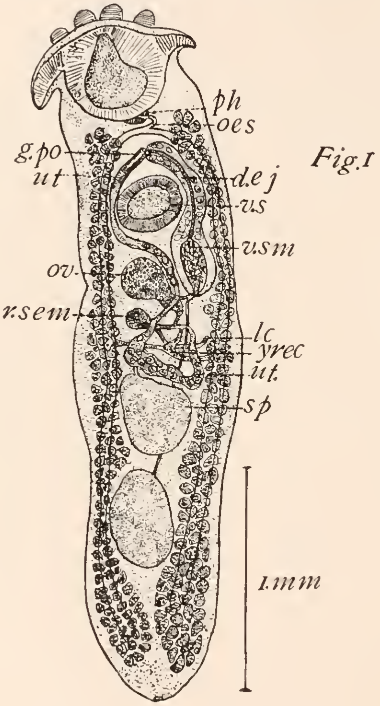

FIG. 1. *B. cornuta*, ventral view, compressed, camera lucida × 30. *d.e.j.*, ductus ejaculatorius; *g.po.*, genital pore; *l.c.*, Laurer's canal; *oes.*, œsophagus; *ov.*, ovary; *ph.*, pharynx; *r.sem.*, seminal receptacle; *sp.*, spermary; *ut.*, uterus; *v.s.*, ventral sucker; *v.sem.*, seminal vesicle; *y.rec.*, yolk receptacle.

The adult stage of *B. cornuta* is found at Chautauqua in the stomachs of black-bass, rock-bass and cat-fish or bull-heads, caught near the Assembly grounds, and earlier stages are found encysted in crayfish, caught near the shore just above the grounds. These localities are near the head of the lake. I have not explored the lake in other places and cannot say how generally the fluke is found in it. The crayfish is clearly the host immediately prior to the fish, as partly digested crayfish are present in the stomachs of fishes where the cysts and the young just escaped flukes are found. The infection of the crayfish is practically universal. The flukes are always found encysted, never free. They are located in the parts immediately related to the reproductive system, most constantly in the muscles, especially those running from the thorax to the abdomen, also in the heart itself, and in the gonads. Remoter organs are not infected. This mode of occurrence indicates that the infection may be through the ducts of the gonads, but I have no observations to decide this point. The number of cysts per individual varies considerably, in one case 40 were found, distributed as follows: 25 in the muscles, 6 in the walls of the heart, 9 in the spermary; in another case: 16 in the muscles, none in the heart or gonad; in still another a few were seen in the muscles and none in the heart or gonads. The cysts vary somewhat in size and structure with the season. In early July they are 0.9 mm. in diameter and consist of a soft fleshy grayish enveloping portion about 0.2

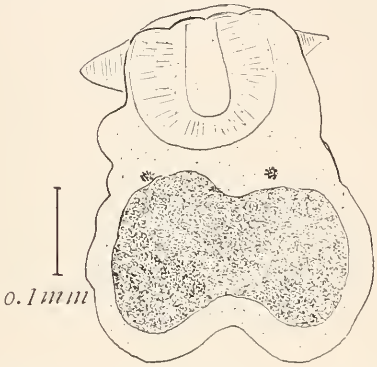

FIG. 2. Young worm still enclosed in cyst, × 120, the shaded area was opaque, and white by reflected light.

mm. thick, enclosing a central mass, dark yellowish-brown and hard, as if perhaps chitinous, of a diameter of 0.5 mm. By manipulating the cysts with little knives made from specially ground needles I found it possible to extract from them a very immature fluke (see Fig. 2) recognizable as *B. cornuta* by its oral sucker. A pair of eyes is present, but the inner organization showed no traces. I suppose the dark granular mass at the posterior end to be a supply of food for the developing worm. Some of the cysts differ by having in place of the hard grandular inner cyst a thin homogeneous covering, enclosing a worm so much more advanced in development, that the alimentary and excretory systems were formed and the genital organs well advanced. In early August cysts having a diameter of 1 mm. or over were found which contained fully matured worms, containing embryos numerous enough to impart a distinctly brown tinge to the parent. These facts are of very considerable interest,

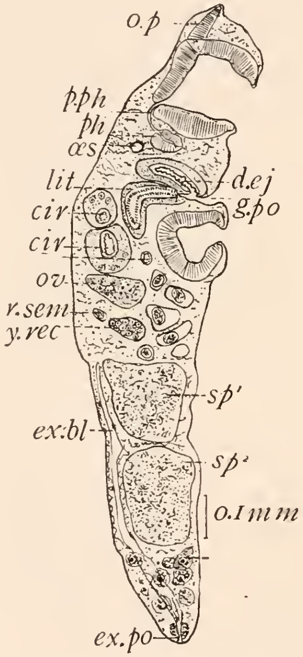

FIG. 3. Sagittal section, cam. luc. × 60. *cir.*, cirrhus ; *ex.bl.*, excretory bladder ; *o.p.*, oral papilla ; *pph.*, pre-pharynx.

for they indicate that the young worm develops actively during encystment, and that here self-fertilization must take place. A fuller study of this point is desirable.

In the fish the parasite has been found only in the stomach. Both cysts separated from the crayfish, and the free worms are found. *B. nodulosa* is reported from the intestine of fishes and *B. auriculata* is also an intestinal parasite.

The body form is nearly cylindrical, in contrast with the elongate neck and almost leaf-shaped body of *B. nodulosa.* This contrast is well seen by comparing Figs. 1 or 7 with Fig. 10 of Looss. The latter is a young stage in which the vitellaria are not as yet developed, while both of the Chautauqua specimens possess them and the uterus contains eggs. My specimens differ considerably in length, owing to the fact that they go on growing longer after maturity. The longest one that I have seen measured 3.0 mm. in length by 0.9 mm. in width (in the preserved and mounted state). I have seen specimens fully developed sexually measuring only 0.9 mm. in length by 0.2 mm. in width. The oral sucker is very large, so that it fills completely the anterior end of the body. It is furnished with remarkable muscular processes, six in number which give the worm a very characteristic appearance. Four of these processes or papillæ are blunt, and extend forward from the dorsal and anterior end of the body. In a ventral view of the animal they are seen extending slightly beyond a thin layer of the body wall which forms the anterior boundary of the body. The other two papillæ are at the posterior level of the oral sucker, and ventral, on the opposite

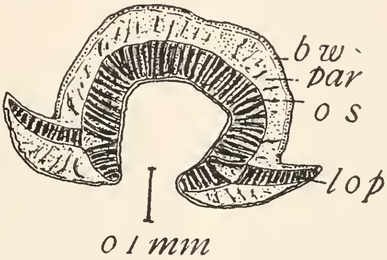

FIG. 4. Transverse section passing through the oral sucker in the level of the lateral processes. Cam. luc. × 50. *l.o.p.*, lateral oral papilla ; *b.w.*, body wall, *par.*, parenchyma ; *o.s.*, oral sucker.

side from the four blunt anterior papillæ, and they are extended transversely to the animal. In form they are tapering and pointed, and slightly curved backward, in the form of a horn, extending considerably beyond the contour of the side of the animal. The oral sucker itself has a diameter of 0.4 mm. The ventral sucker, while large, is smaller than the oral sucker, its diameter being 0.3 mm. Its position in Fig. 1 is strikingly far forward; in Fig. 7 it is more nearly in the center of the body. This difference is due to contraction of the neck in Fig. 1, shown also by the winding course of the œsophagus of that specimen. The genital pore is located in front of and near to the ventral sucker, in the middle line. Eyes are present in younger specimens, but older ones do not possess them, though in these it is possible to find scattered grains of pigment in the region of the pharynx, indicating their late disappearance. Looss represents eyes in both the specimens figured by him, so that if they are not exceptional cases, the eyes persist to a much later time in the life history of *B. nodulosa* than of *B. cornuta*.

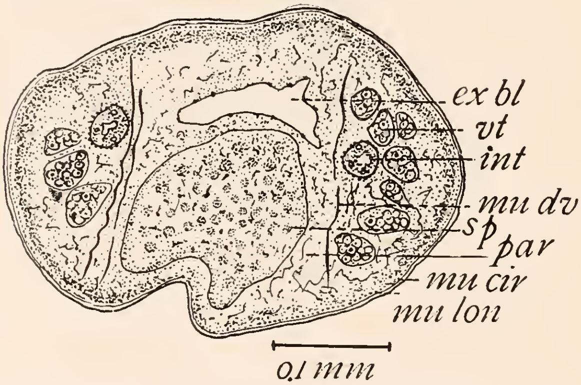

FIG. 5. Transverse section in level of the posterior spermary. Cam. luc. × 115. *vt.*, vitellaria; *mu.d.v.*, dorsiventral muscle; *mu.cir.*, circular muscle of the body wall; *mu.lon.*, longitudinal muscle of the body wall.

The body wall presents the usual cuticle, destitute of spines. The usual muscle layers are present, the fibers of the outer circular layer are very fine indeed; those of the longitudinal and oblique layers are exceptionally large. Parenchymatous muscle is somewhat specially collected in each side of the body running dorsoventrally, and marking off a lateral area, containing the vitellaria and the intestine, from the center (see Fig. 5). There are no horizontal parenchyma muscles. Cells of the parenchyma directly underlying the body wall are especially numerous and glandular in appearance, as often in trematodes.

The oral sucker opens widely downwards and forwards. It is composed of the usual muscular masses, enclosed within a fine structureless membrane marking it off from the parenchyma. The detailed structure of the papillæ is indicated in Fig. 4, which is a camera lucida drawing from a section passing through the oral sucker in the level of the lateral papillæ and through their length. Sections through the four anterior papillæ show the same things. The wall of the oral sucker, consisting of a layer of parenchymatous tissue and masses of radial fibers, is pushed out at the bases of the papillæ, the connective tissue portion being directly continuous and the muscle fibers pushed aside at that point, and passes up to the summit of the papilla, a new set of radial fibers being added in the papilla similar to those of the general wall of the sucker. The papillæ are thus not merely

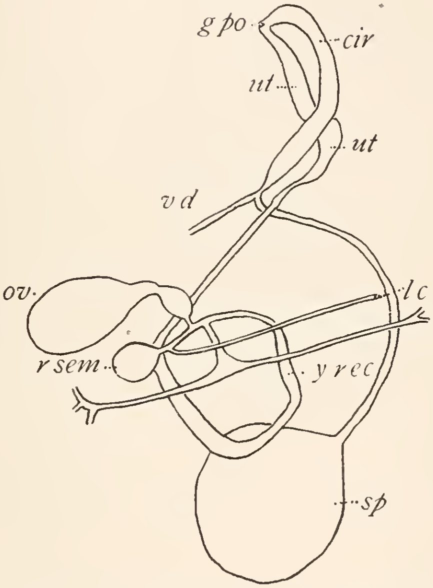

Fig. 6. General view of the reproductive organs, seen dorsally.

surface features of the animal, but deep-seated in their origin, and are entitled to be regarded as of considerable importance from a taxonomic point of view.

The lateral papillæ are unmistakable organs not likely to be overlooked by an observer, whereas the ventral papillæ of *B. nodulosa* are inconspicuous and might easily escape notice, a point discussed by Looss ('94, p. 34). In *B. auriculata* I looked very closely for the dorsal papillæ without finding them and I am convinced that they are absent from the specimen I saw, in which respect my observations confirm those of Linton as indicated in his figures, '98, Pl. XLV., Figs. 1–7.

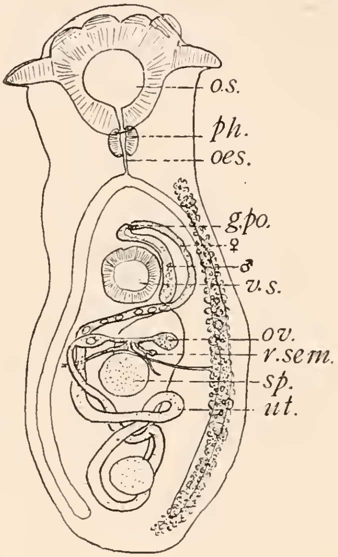

FIG. 7. Ventral view of a specimen in which the uterus is most fully developed, the vitellaria are omitted from the right side.

There is a short pre-pharynx, a small pharynx, about 0.05 mm. long, a short œsophagus, its walls very strongly muscular and surrounded by glandular cells. The forking of the intestines is thus close to the pharynx, a point different from *B. nodulosa.* The intestines are simple and long, reaching to near the hinder end of the body. They are lined with epithelium cells whose outer ends are elongate and whose tips extend into the cavity of the organ. Circular and longitudinal muscle fibers are present in the wall.

The excretory pore is terminal. Close in front of it is the excretory bladder, which in sections can be seen running dorsally forward at least as far as the level of the front of the anterior testis (see Figs. 2, 5). I have not been able to recognize more than the most posterior portion in living animals. According to Looss there is in *B. nodulosa* a bladder wholly posterior to the hinder testis containing concretions, and from which vessels run forward on either side. I have not seen such concretions in *B. cornuta* and the bladder is much more extensive than that.

There are two large testes, 0.3 mm. across, lying one directly in front of the other. In *B. nodulosa* the testes are smaller, farther apart and oblique. The testes are crowded with active sperm cells, many of them in the last stages of spermatogenesis, and with numerous fully formed spermatozoa. The seminal vesicle and spermatic receptacle are also filled with them.

Long and slender vasa deferentia run dorsally to the other genital organs, and meet at the posterior end of the large cirrhus sack which is located some distance behind the posterior border of the ventral sucker. The cirrhus sack is very large indeed, much larger than in *B. nodulosa.* It has a definite outer wall, strongly muscular, enclosing a tubular passage subdivided into two portions, a posterior thin-walled part, the seminal vesicle, and an anterior ductus ejaculatorius. This latter is surronded by glandular "prostate" cells, is very strongly muscular, having both circular and longitudinal fibers. The ductus ejaculatorius is not coiled. I do not know whether it is eversible or not.

The ovary is generally located on the right side, but not infrequently it is found on the left (cf. Figs. 1 and 7). It is always near the ventral sucker, a large and conspicuous organ. There is a short ciliated oviduct, soon joined by first a duct from the seminal receptacle, then one from the yolk receptacle. Certain glandular-looking cells which lie around the oviduct may perhaps represent a shell gland, but a distinct and well-marked organ is not present. Nearly all of my specimens appear to be quite young, and though the uterus contains eggs it is not fully developed. In one, however (Fig. 7), the uterus is longer and evidently more as in fully matured individuals. In this case the uterus is distinctly tubular and winds down and back, passing between the testes in its course, in *B. nodulosa* the uterus is a large sack containing old and young eggs indiscriminately, the uterus is saccular even in young individuals of *B. nodulosa*, as seen in Looss' Fig. 10 of a specimen before egg production has begun. The terminal part of the uterus differs decidedly from the rest so as to form an entirely distinct though continuous organ (cf. Fig. 2.) Its wall is very thick indeed and consists of a strong muscular coat quite unlike the wall of the deeper parts of the tube, and within the wall is furnished with a clothing of very peculiar numerous long slender bluntly ending processes. which are free at tip in the cavity of the organ. These structures do not look like cilia, being too blunt. They do not seem certainly to be protoplasmic, at least the bases do not seem — as far as I have been able to study them — to be nucleated cells, as we should expect. The histological structure of this part will have to be left for a subsequent study. This organ is further surrounded by parenchyma cells having much the same appearance as the prostate cells of the cirrhus. The eggs measure 0.07 mm. in length instead of 0.1 mm. as in *B. nodulosa.*

Laurer's canal is present, passes dorsally and opens to the exterior on the left side. The seminal receptacle is large and distinct; it lies close to and just behind the ovary. It is in all cases of adults filled with spermatozoa. The vitellaria are large, and located as above described laterally and so as partly to envelope the intestines. They extend from near the phaynx to near the hind end of the body and consist of very numerous small follicles uniformly distributed. A duct from each crosses the body in front of the anterior testis and behind the ovary and seminal receptacle and the two joining from the yolk receptacle which reaches the oviduct by a short duct close to the ovary.

The points made in the foregoing pages are summarized in the following table of comparisons :

	B. cornuta.	*B. nodulosa.*
Total length,	3 mm.,	3 mm. Looss, 4.5 mm. Olsson.
Body form,	cylindrical,	leaf-shaped.
Neck,	not prominent,	prominent and distinct.

Lateral papillæ,	transverse and hook-shaped,	longitudinal and blunt, not hook-shaped.
Eyes,	not persistent,	persistent in adult.
Œsophagus,	short,	long.
Excretory bladder,	long,	short.
Testes,	close together in median line,	wide apart, and oblique.
Uterus,	tubular,	saccular.
Ova,	0.07 mm. long,	0.1 mm. long,
Residing,	in stomach,	in intestine of host.

Biological Laboratory, Hamline University,
Saint Paul, Minn., March 10, 1903.

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ON THE BLOOD VESSELS, THEIR VALVES AND THE COURSE OF THE BLOOD IN LUMBRICUS.[1]

J. B. JOHNSTON.

In a previous paper[2] an account has been given of the experimental study of the course of the blood flow in *Lumbricus* The most important result there set forth was that the circulation is not segmental but strictly systemic. The course of the flow is as follows : forward in the dorsal vessel for its whole length ; downward in the hearts ; both forward and backward from the hearts in the ventral vessel ; outward from the ventral to the body wall, nephridia and intestinal wall ; toward the lateral neurals from the body wall ; backward in the subneural ; upward to the dorsal vessel in the parietals from the subneural, the nephridia, and the body wall, and in the dorso-intestinals from the intestine. Thus, there is no circuit of blood in each segment to which a sytemic circuit for part of the blood has been superadded, as all previous authors have maintained, but all of the blood flows in a single systemic circuit. In the head region the blood is carried forward beyond the hearts by both dorsal and ventral vessels and is returned to the dorsal behind the hearts in larger part by the lateral œsophageals, and in smaller part by the subneural and the parietals of XII. and succeeding somites. The lateral œsophageal system is considered to represent the parietals in the somites anterior to XII.

This view of the circulation raised two important questions which further work has answered : (1) What happens when the hearts are removed from the circulation by decapitating the worm ? Do the conditions which obtain in the regenerating worm confirm the above results? (2) What is there in the structure of the blood vessels to determine and control the course of the blood?

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[1] Studies from the Zoölogical Laboratory of West Virginia University, No. 8, February 28, 1903. A part of the work reported here was done by my former student, Miss S. W. Johnson. For the conclusions reached the present writer alone is responsible.

[2] " The Course of the Blood Flow in *Lumbricus*," by J. B. Johnston and Sarah W. Johnson, *Amer. Naturalist*, April, 1902.

The first question has been answered by a series of regeneration experiments carried out upon large and small specimens of *Lumbricus*. Operations removing from eleven to twenty somites from the anterior end were performed upon 171 worms. These were examined alive from time to time and eventually 20 were hardened for sectioning. The time that the worms were allowed to live varied from ten days to three and a half months. In a few worms regeneration progressed well, but the majority died after a few days or weeks. A detailed report upon these experiments would not be profitable for our present object. Although there were very great variations in the condition of the blood vessels, the following may be said to be true in greater or less degree of all the worms studied alive or sectioned. The vessels in the anterior one fourth to one half of the worm were greatly crowded and distended with blood. The anterior portion of the worm was usually a bright red to the naked eye and under a lens many small vessels not usually visible were distinctly seen. Sections showed that all the vessels were more or less crowded with blood, while the dorsal, subneural, and the vascular plexus of the intestinal wall showed the greatest distension. The ventral vessel was seldom stretched much beyond its normal size, while the subneural was often as great in diameter as the ventral. Occasionally the subneural was much larger than the ventral and sometimes its cross-section was equal to that of the nerve cord. In several cases the vascular layer of the intestine was very greatly crowded and, considering its great capacity in normal conditions, it is probable that it always held the greatest accumulation of blood. The posterior portion was very poor in blood in all worms.

These conditions are readily explained in accordance with the scheme of circulation above summarized. The fulness of the dorsal, intestinal and subneural vessels is due to the pressure from the dorsal which is deprived of the normal outlet for the blood carried by it, and forces the blood downward in the dorso-intestinals and parietals contrary to its usual course. The small amount of blood in the ventral is due to the absence of the hearts and the inability of the dorsal to drive the blood through the capillary systems to the ventral. The absence of blood in the posterior end is a further result of the small amount of blood received by the ventral. If there were a segmental circulation in *Lumbricus* there would probably be no great accumulation of blood at the anterior end in these experiments, since the segmental circulation would tend to relieve the systemic and the even distribution of the blood would be maintained in accordance with the law of least resistance. These regeneration experiments,

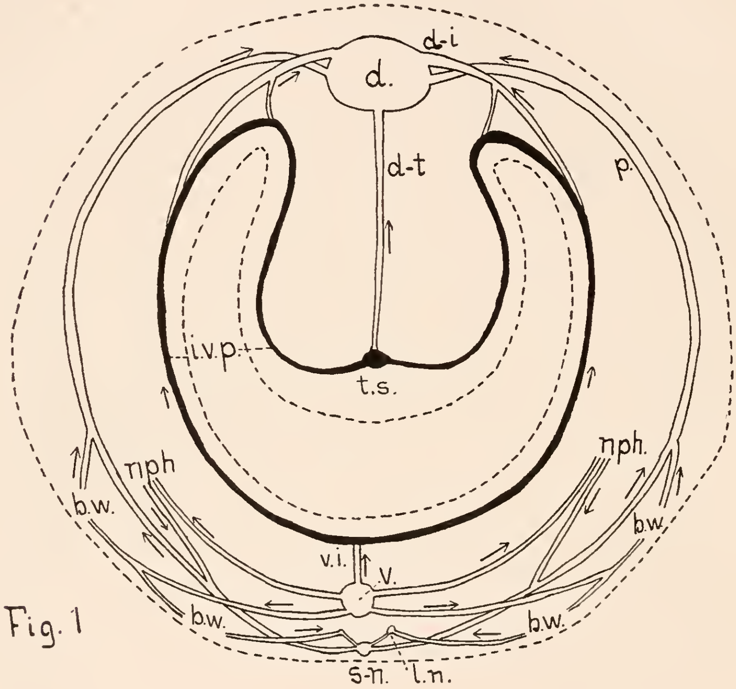

Fig. 1

FIG. 1. General scheme of circulation in body region, all the vessels of one segment being projected upon the plane of a transverse section. The vascular layer of the intestine is shown by a broad black line. In the typhlosole the vascular plexus thickens at one place to form the typhlosolar sinus which varies greatly in size in different worms and in different parts of the same worm. From this sinus three dorso-typhlosolar vessels in each segment carry blood to the dorsal vessel. These vessels and the branching of the dorso-intestinal vessels shown in this figure have not before been correctly described or figured for *Lumbricus*.

therefore, seem to confirm the results of physiological experimentation. No effort was made to trace the development of hearts in the regenerated heads and the final reorganization of the circulation, and it is doubtful whether the worms would have lived long enough for this purpose.

It is probable that the failure of the blood vessels to adjust themselves to the new conditions is at least one of the chief causes of the death of worms under these experiments. The continued strong pulsations of the dorsal vessel after the removal of the hearts force the blood out through vessels which normally empty into it. In some cases the reversal of flow through the vessels of the body wall and intestines is produced readily enough to allow the worm to survive the operation, but in most cases less blood would reach the ventral vessel than is necessary to supply the posterior end of the worm and an insufficient amount of blood would pass through the respiratory plexus beneath the hypodermis. The blood which leaves the dorsal vessel in the anterior part of the worm either settles in the vascular layer of the intestine, which readily expands to receive it, or passes directly through the parietals to the subneural, which is consequently greatly expanded; and avoids the respiratory plexus because of the resistance in that quarter. A similar withdrawal of blood from the respiratory plexus of the posterior end of the worm also results indirectly from the small amount of blood in that region, so that the whole worm is seriously deprived of needed oxygen. In the normal circulation the blood is driven to the respiratory plexus from the ventral under direct pressure from the hearts, and there is no other way of less resistance by which the blood may return from the ventral to the dorsal. Upon the view of the circulation held by Bourne[1] and Harrington,[2] namely, that the dorso-intestinals empty into the dorsal vessel and the parietals carry blood away from it, it is evident that the path of least resistance from the pulsating dorsal vessel is through the parietals directly to the subneural and that there would be nothing to drive the blood through the respiratory

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[1] Bourne, A. C., "On *Megascolex cœruleus* and a Theory of the Course of the Blood in Earthworms," *Q. J. M. S.*, Vol. 32, p. 49, 1891.

[2] Harrington, N. R., "The Calciferous Glands of the Earthworm, with an Appendix on the Circulation," *Jour. Morph.*, Vol. 15, Suppl., p. 105, 1899.

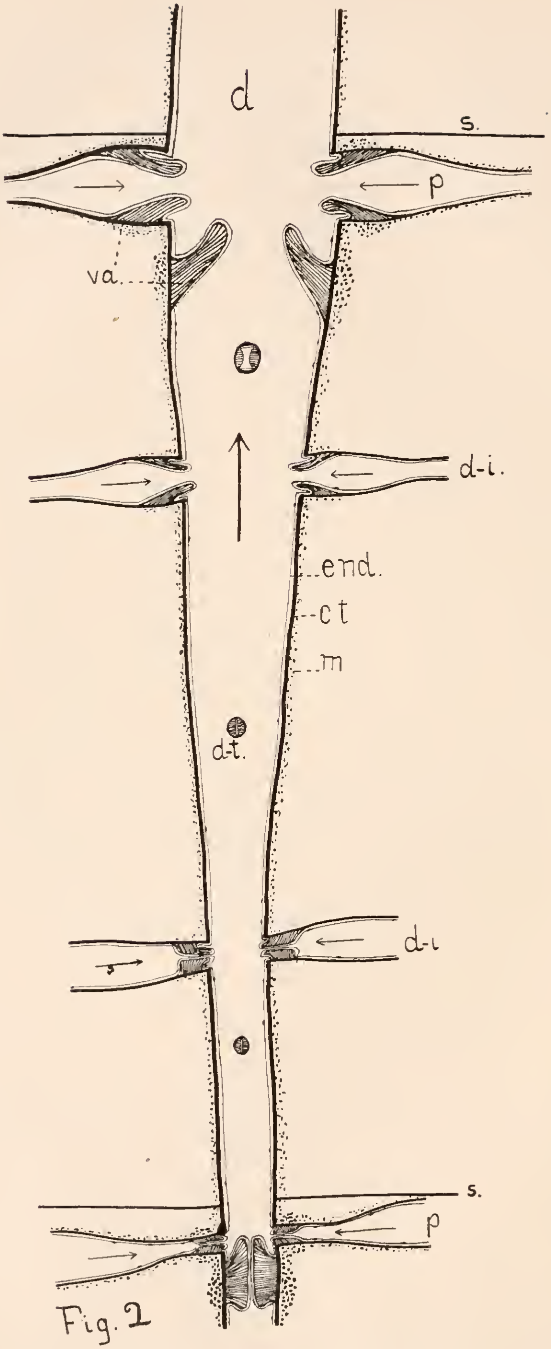

Fig. 2. A diagrammatic horizontal section of the dorsal vessel and those emptying into it. One somite and part of a second are shown, and the last part of a pulse wave and the greater part of a following contraction wave are represented. The arrows show the course of the blood and the position and changes of form of the valves are shown. The chloragogue cells covering the walls of the vessels are not drawn.

capillaries of the body wall. This is perhaps an insuperable objection to that theory of the circulation. This objection does hold against the view of Perrier[1] and Benham,[2] according to which the blood flows to the dorsal vessel in the parietals and out from it in the dorso-intestinals.

The study of the structure of the vessels shows that the movement of the blood is determined by the structure of the walls and by definite valves which several of the vessels possess. The wall of the dorsal vessel consists (Fig. 2) of a lining endothelium of very thin cells whose nuclei alone are usually visible; a connective tissue layer containing a few longitudinal (muscle?) fibers, and a well-developed layer of circular muscle fibers. Outside these are the chloragogue cells. To the layer of circular muscle fibers are due the pulsations of the dorsal vessel, and thickenings of this layer at certain points assist in the action of the valves, as will be described below. The wall of the ventral vessel has no circular muscle layer. Its lining endothelium is more conspicuous than that of the dorsal vessel and the connective tissue layer is very thick. This is a strong fibrous layer and gives great rigidity to the wall of the ventral vessel. Outside of the connective tissue layer are a few (4 to 6) strands of longitudinal fibers which take the same stain as the muscle fibers in the sheath of the neighboring nerve cord. Outside these fibers is a layer of peritoneum closely similar to that covering the inner surface of the body wall.

The subneural consists of only the endothelium and connective tissue layer, outside of which is the sheath of the nerve cord. This is the structure of the lateral neurals also, and of all the smaller vessels. The dorso-intestinals and parietals present an intermediate condition between those with and those without a circular muscle layer. The dorso-intestinal vessels are devoid of muscle fibers except at their dorsal ends where there is a thin extension of the circular layer of the dorsal vessel for a short distance. The parietals are provided with a thick band of circular fibres close to their connections with the dorsal and the layer is continued along the vessel for about a third or half its length. These muscle fibers in the dorsal portion of the parietals produce the active pulsations which have been described in an earlier paper (*loc. cit.*, p. 323).

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[1] Perrier, Edw., "Recherches pour servir à l'histoire des Lombriciens terrestres," *Nouv. Arch. du Mus. d'Hist. Nat.*, Paris, Tome 8, 1872.

[2] Benham, W. B., "The Nephridium of *Lumbricus* and its Blood Supply," *Q. J. M. S.*, Vol. 32, p. 293, 1891.

The walls of the hearts have the same structure as that of the dorsal vessel, except that they are covered with chloragogue cells only in their dorsal portion, elsewhere by peritoneum. The circular muscle fibers are large and the layer somewhat stronger than that in the dorsal vessel.

The structure of the vessels determines whether they shall propel the blood by their pulsations or only carry it, and the account of the structure accords with the well-known facts concerning the pulsations of the vessels. Pulsations in the dorsal, parietals and hearts are well established; pulsations in other vessels, described by Harrington, have not been seen by the author and to whatever extent they occur they must be produced without muscle fibers.

Valves are present in the dorsal vessel and in all the vessels connected with it, namely, the dorso-intestinals, dorso-typhlosolars, parietals, lateral œsophageals (?) and hearts. The valves in the dorsal are a pair of large thick flaps attached to the lateral walls of the vessel at a point a short distance behind each septum and immediately behind the openings of the parietals. These valves are always directed forward and allow the free passage of blood during the pulse wave. As the contraction wave approaches, the valves are brought into contact and at the moment of greatest constriction the two flaps are tightly pressed together and completely close the lumen of the vessel. The efficiency of the valves is secured and increased by a considerable thickening of the circular muscle layer at the valve (Fig. 2). The valves do not act in the ordinary manner of flap valves, but the two fleshy flaps are pressed together and form a large mass which fills the vessel. In the region of the hearts a pair of valves is found in the dorsal vessel a short distance in front of each pair of hearts.

The valves in the dorso-intestinal, dorso-typhlosolar and parietal vessels are essentially the same in form and position. In each of these vessels (Fig. 2) a pair of small fleshy flaps are situated at the opening of the vessel into the dorsal. In the dorso-intestinal and parietal vessels the flaps are attached one to the anterior and one to the posterior wall of the vessel, and the body of the flap projects into the lumen of the dorsal vessel. In the dorso-typhlosolars the flaps are lateral in position, are situated deeper in the vessels, and do not project so far into the dorsal. It is evident that pressure from the dorsal toward any of these vessels would tend to close the valves. The closing of the parietals is further secured by a thickening of the circular muscle layer as in the dorsal; and in the dorso-intestinals a thin extension of the muscle layer of the dorsal serves the same purpose. Muscle fibers have not been observed in the dorso-typhlosolar vessels. The valves in these vessels allow the blood to flow from them into the dorsal only, and this accords with the results obtained by the earlier experimental investigation. In the case of the decapitated worms the valves in all these vessels near the anterior end must have been forced.

The hearts are better supplied with valves than are any of the other vessels. In each heart are four pairs of valves (Fig. 3); one situated close to the dorsal vessel, one between the first and second thirds from the dorsal end, one between the second and third thirds, and the fourth in the ventral end of the heart at the opening into the ventral vessel. The three pairs in the body of the heart are like those in the dorsal vessel but are smaller in proportion to the diameter of the heart. They are inclined downward and are large enough to close the heart during its contraction. The presence of these valves might seem unnecessary in view of the fact that the contraction waves pass along the heart from above downward. However, if from any cause the contraction becomes modified or irregular or if the whole heart contracts at once, the functional importance of these valves is evident. It is a matter of common observation that such irregularities in the contractions of both the hearts and the dorsal vessel do appear in worms dissected alive under an anæsthetic, and it is probable that such irregular contractions and the influence of movements of the body make necessary the valves in the hearts and the dorsal vessel in the normal worm. The valves in the smaller ventral ends of the hearts fill the lumen and project into the ventral vessel very much as the valves in the parietals project into the dorsal. Thus, with the valves in the dorsal between each two pairs of hearts and the four valves in each heart, regurgitation of blood during the strong cardiac contractions is effectively guarded against.

The study of the fine structure of the valves has presented great difficulties because methods of fixation which give satisfactory preparations of all other tissues give very imperfect pictures of these valves. This itself indicates one fact regarding their structure, namely, that they are composed of very soft-bodied or watery cells which may appear vacuolated or shrunken, or even macerated. In many preparations the valves appear only as masses of granular or coagulated material containing many ovoid nuclei. In the most successful sections, however, the valves

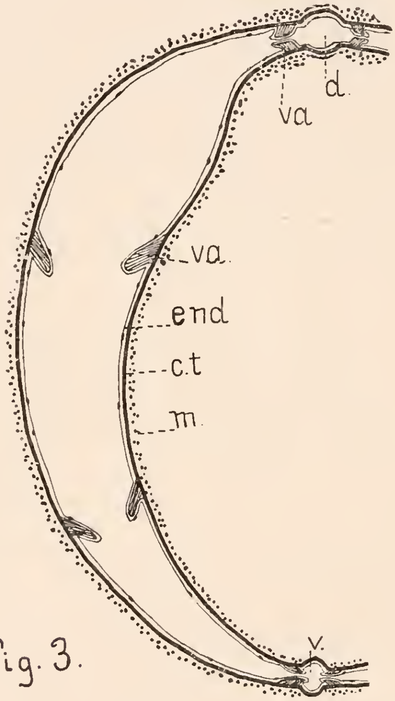

Fig. 3.

FIG. 3. A diagrammatic cross-section through one of the hearts to show the position of the valves. The chloragogue and peritoneal epithelium are not drawn.

show indistinct cell boundaries which produce an appearance of striations running from base to free edge of the valve. In most preparations, especially in longitudinal sections of the dorsal vessel, which are often oblique owing to the curves of the vessel, the substance of the valves appears to be sharply delimited from the connective tissue layer. This would indicate that the valve is formed by a thickening of the endothelial layer. It is difficult to disprove this first supposition because the endothelial cells are so broad that one can seldom expect to find an endothelial nucleus on the surface of a valve. However, in some cases in the hearts flattened nuclei similar to those of the endothelial cells are found on the surface of the valves. Cross-sections of the dorsal vessel through the base of the valves show a radial striation running from the valves through the connective tissue and muscle layers. From these facts it appears that the valves are composed of elongated cells which run through the connective tissue layer and securely anchor the valves. Since they are covered internally by endothelial cells they must be regarded as belonging to the connective tissue layer. Essentially the same structure is presented by all the valves, although those in different positions differ greatly in size and form in relation to the function which they have to perform. The largest valves are those in the dorsal vessel. These are thick flaps attached by broad bases to the lateral walls of the vessel. When the vessel is distended the valves are nearly semilunar in form. When the vessel is contracted the valves become greatly compressed against one another and the soft substance of the valve is forced both forward and backward from its point of attachment. When the valve extends far forward it overlaps the opening of the parietal vessel and might appear to function to close that vessel. Such a condition seems to have been seen by Benham (*loc. cit.*). The valves in the dorso-intestinal and parietal vessels are also paired flaps, but owing to the small size of the vessels the flaps are small at their bases and are longer than they are broad. Often these valves have a balloon form as they project into the dorsal vessel. The valves in the dorso-typhlosolar vessels are situated somewhat farther within the vessels and are more nearly simple semicircular flaps. The valves at the ventral ends of the hearts are relatively large and project so far into the ventral vessel that they might be mistaken for valves proper to the ventral vessel itself.

The course of the blood flow is determined by the disposition of the valves as well as by the direction of the pulsations, and there is evidently entire agreement between the results of the physiological experiments and anatomical investigation. It is obvious that in small vessels or in such as receive blood from a capillary system so that there is no great pressure in the usual course, there may occur temporary reversals of flow due to movements of the body or other causes. Such reversals might most readily take place in the subneural vessel and such phenomena are probably the basis for Harrington's statement that the blood flows now forward, now backward in the subneural. However, the general course of the blood flow is strictly determined, as shown by the consistent experimental and anatomical results, and no considerable or long-continued reversal or interruption of the usual current are possible except as the result of violent interference such as decapitation of the worm.

The valves in the vessels have received very meager notices heretofore. The mention of valves in the dorsal vessel by Benham has been noticed above. A recent writer[1] has mentioned the presence within the dorsal vessel of cells similar to the chloragogue cells. These are also doubtless the valves of the dorsal vessel.

Explanation of Figures.

Abbreviations: b.w., body wall; *c.t*, connective tissue layer of blood vessels; *d.*, dorsal vessel; *d-i.*, dorso-intestinal vessel; *d-t.*, dorso-typhlosolar vessel; *end.*, endothelial lining of vessels; *i.v.p.*, vascular plexus of intestine; *l.n.*, lateral neural vessel; *m.*, layer of circular muscle fibers in walls of vessels; *nph.*, nephridium; *p.*, parietal vessel; *s.*, septum; *s-n.*, subneural vessel; *t.s.*, typhlosolar sinus; *v.*, ventral vessel; *va.*, valve; *v.i.*, ventro-intestinal vessel.

[1] Rice, Wm. J., "Studies in Earthworm Chloragogue," BIOL. BULL., Vol. III., Nos. 1–2, 1902.

TWO NEW GENERA OF MALLOPHAGA.

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There have come to me recently specimens of Mallophaga, taken from birds from mid-ocean islands, which demand the founding of two new genera in this interesting but little-studied order of parasitic insects. In the order, as at present known, there are about 1,500 species, comprising twenty-three genera. The small number of genera is striking in itself, but is made more amazing when it is remembered that eleven of the genera comprise but thirty of the species, leaving thus nearly the whole bulk of the species included in the twelve remaining genera. The addition of two new genera is, therefore, rather notable in the development of our systematic knowledge of the group. Although about two hundred new species of Mallophaga have been described from North American birds but one new genus (my *Giebelia*, with only one species, from shearwaters) has had to be established, all the other North American species being referable to genera founded on Old World species and specimens. The following revised key to the known genera of the order (including the two new genera described in this paper) is presented for the use of beginning students of the group, or of general entomologists :

Analytical Key to Suborders of Mallophaga.

With filiform, 3- or 5-segmented, exposed antennæ ; no labial palpi ; mandibles vertical ; œsophageal sclerite and accompanying glands usually present and normal ; meso- and metathoracic segments fused ; crop a saclike diverticulum ; ingluvial glands present ; testes four ; egg tubes five............................. Ischnocera.

With clavate or capitate, 4-segmented, concealed antennæ ; with 4-segmented labial palpi ; mandibles horizontal ; œsophageal sclerite and accompanying glands absent or modified ; meso- and metathoracic segments with sutural line usually visible ; crop simple ; ingluvial glands absent ; testes six ; egg tubes three to five. Amblycera.

Analytical Key to Genera of the Suborder Ischnocera.

A With 3-segmented antennæ ; tarsi with one claw ; infesting mammals (family Trichodectidæ)... *Trichodectes* Nitzsch.

AA With 5-segmented antennæ ; tarsi with two claws ; infesting birds (family Philopteridæ).

B Antennæ similar in both sexes.
C Meso- and metathoracic segments not fused........... *Nesiotinus* Kellogg.
CC Meso- and metathoracic segments fused.
D Front deeply angularly notched.................. *Akidoproctus* Piaget.
DD Front convex, truncate, or rarely with a curving emargination, but never angularly notched.
E Species broad and short, with large, movable trabeculæ (at the anterior angle of antennal fossa).
F Forehead with a broad transverse membranous flap projecting beyond lateral margins of the head in the male, barely projecting in the female......... *Giebelia* Kellogg.
FF Without such membranous flap...... *Docophorus* Nitzsch.
EE Species elongate, narrow, with very small or no trabeculæ. *Nirmus* Nitzsch.
BB Antennæ differing in the two sexes.
C Species wide, with body elongate-oval to suborbicular.
D Temporal margins rounded; last segment of abdomen roundly emarginated; antennæ of male without appendage; third segment very long............................. *Eurymetopus* Taschenberg.
DD Temporal margins usually angulated; last segment of abdomen convex, rarely angularly emarginated, with two points.
E First segment of antennæ of male large, sometimes with an appendage; third segment always with an appendage. *Goniodes* Nitzsch.
EE First segment of antenna of male enlarged, but always without appendage; third segment without appendage; last segment of abdomen always rounded behind. *Goniocotes* Nitzsch.
CC Species elongated narrow, sides subparallel.
D Third segment of antenna of male without an appendage. *Ornithobius* Denny.
DD Third segment of antenna of male with an appendage.
E Front deeply angularly notched.. *Bothriometopus* Taschenburg.
EE Front not angularly notched.
F Forehead with a broad transverse membranous flap or fold projecting beyond lateral margins of the head. *Philoceanus* Kellogg.
FF Without such membranous flap.
G Antennæ and legs long; a semicircular oral fossa. *Lipeurus* Nitzsch.
GG Antennæ and legs short; oral fossa narrow, elongate, extending as a furrow to the anterior margin of the head.............................. *Oncophorus* Rudow.

ANALYTICAL KEY TO GENERA OF THE SUBORDER AMBLYCERA.

A Tarsi with one claw; infesting mammals (family Gyropidæ)... *Gyropus* Nitzsch.
AA Tarsi with two claws; infesting birds (excepting *Boopia*?) (family Liotheidæ).
B Ocular emarigination distinct, more or less deep.
C Forehead rounded, without lateral swelling; antennæ projecting beyond border of the head .... *Colpocephalum* Nitzsch.

CC Forehead without strong lateral swellings.

D Antennæ projecting beyond border of the head ; temporal angles projecting rectangularly ; eye large and simple..... *Boopia* Piaget.

DD Antennæ concealed in groove on under side of the head ; temporal angles rounded or slightly angular ; eye divided by an emargination and fleck.

E Mesothorax separated from metathorax by a suture. *Trinoton* Nitzsch.

EE Meso- and metathorax fused ; no suture. *Læmobothrium* Nitzsch.

BB Ocular emargination absent or very slight.

C Sides of the head straight or slightly concave, with two small projecting labral lobes.. *Physostomum* Nitzsch.

CC Sides of the head sinuous ; forehead without labral lobes.

D Ocular emargination filled by a strong swelling ; sternal markings forming a quadrilateral without median blotches.. *Nitzschia* Denny.

DD Ocular emargination without swelling, hardly apparent or entirely lacking ; median blotches on sternum.

E Very large ; with two 2-pointed appendages on ventral aspect of hind head ; anterior coxæ with very long lobe-like appendages .... *Ancistrona* Westwood.

EE Small or medium ; without bipartite appendages of hind head .... *Menopon* Nitzsch.

PHILOCEANUS gen. nov.

In a collection of Mallophaga taken by Mr. Rollo Beck from birds of the Galapagos Islands (the collecting of birds and parasites was done by Mr. Beck in the summer of 1901), are five specimens, including one male, three females, and one young, from a single specimen of *Procellaria tethys* (Wenman Id.) of a Mallophagan species not assignable to any of the known genera. The shape and habitus of whole body and the secondary structural differences between the sexes, shown in antennæ and abdominal segments, are those of *Lipeurus*, while the well-developed and unusual transversal membranous clypeal flap is that of *Giebelia*. The curious prolongation of the postero-lateral angles of the mesothorax is a character peculiar to the new genus. As *Giebelia* with its short, broad body and antennæ similar in both sexes stands to *Docophorus*, so the new genus, which may be called *Philoceanus*, with its elongate body, and differing antennæ, stands to *Lipeurus*.

The characteristics of the new genus may be given as follows : body Lipeuroid, elongate ; head, thorax and abdomen of about equal width (in widest places); antennæ differing in the sexes, that of male having an appendage on third segment; abdomen of male narrower than in female, parallel-sided, and with segments 6–8 each about twice as long as each of preceding segments; head with a broad, thin, transvenal, membranous clypeal flap projecting far on each side of forehead in an angulated and folded process; metathorax with postero-lateral angles conspicuously produced into tapering, blunt-pointed, backward-projecting processes.

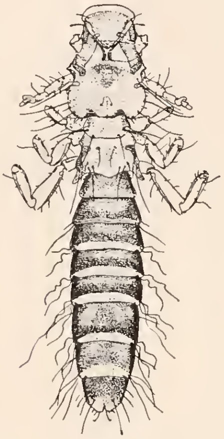

FIG. 1. *Philoceanus becki*, male. (Length, 1.6 mm.

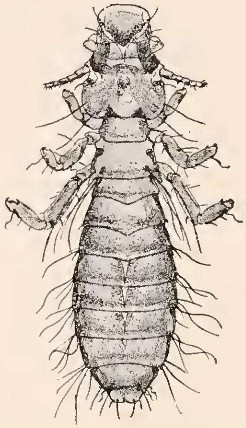

FIG. 2.) *Philoceanus becki*, female. (Length, 1.5 mm.)

Philoceanus becki sp. nov. (Figs. 1 and 2.)

Five specimens (one male, three females, one immature) taken from *Procellaria tethys* (one specimen) Wenman Id. of the Galapagos group, summer of 1901, by Mr. Rollo Beck.

Description of Male.—Body, length 1.6 mm., width .27 mm. (abdomen), pale yellowish brown, with darker to blackish-brown marginal and transverse bands which cover so much of the surface as to give the posterior half of the body a general dark brown coloration.

Head, length .4 mm. width .3 mm., large in comparison with rest of body, wider than any other part of body, and conspicuously large, *i. e.*, wider and longer than the thorax; clypeal front broad, flatly convex and with distinct thin uncolored rounding margin; clypeal sutures distinct, broad, and with two conspicuous hairs at the marginal termination; these clypeal sutures form a V-shaped figure enclosing the distinct clypeal signature between the anterior prongs; the clypeus bears a conspicuous membranous flap or fold, thin and uncolored, which rises from about in transverse line with the mandibles and projects forward to the point of the clypeal sutures, and laterally conspicuously beyond the margins of the head; in these lateral extensions the flap is folded back (towards the head margin) on itself; eye with rather long hair; the temples are not much swollen and each bears two long and a few short hairs; the antennæ (Lipeuroid) have the first segment as long as all the others combined and the third segment with an appendage; the ground color of the head is pale translucent yellowish-brown with the clypeal signature, a broad submarginal angulated band on each side of head, extending from clypeal suture to base of antennæ, darker brown.

Thorax small; prothorax with rounded postero-lateral angles with two separated longish hairs in each; metathorax a little wider and about twice as long, with postero-lateral angles conspicuously produced as thick, tapering, blunt pointed, finger-like processes, a long hair rising from base of each process and another not so long and two or three short ones rising from general postero-lateral angular region; posterior margin of metathorax slightly angulated in the middle and slightly concave in the space between this median angle and the postero-lateral angle; color pale translucent yellowish-brown with darker rather broad lateral margins.

Abdomen elongate, rather narrow, subparallel-sided; segments 1–5 each about one half as long as segments 6–8; long, flexible curling hairs in postero-lateral angles of segments 2–7, and terminal segment with many short fine hairs; pale yellowish-brown ground color almost wholly obscured by strong dark to blackish-brown lateral and transversal bands.

Female. — About same size as male but with abdomen wider (.4 mm.) in the middle and thus not parallel-sided; ground color of whole body less pale and translucent than in male; head with transversal clypeal flap as in male; antennæ without appendage on third segment and with first segment shorter than second; thorax with postero-lateral finger-like processes of meta-segment and with three or four long hairs in postero-lateral region; abdomen with second segment longest, others about equal among themselves, and segments 4–6 (in middle of abdomen) wider than others, so that the whole abdomen is elongate elliptical in outline; last segment with slight angular median emargination on posterior margin.

NESIOTINUS gen. nov.

A single female Mallophagan specimen of well-defined character received from Dr. G. Enderlein, of Berlin, proves to be a form which it is impossible to ascribe to any known genus of the order. This specimen was taken from *Aptenodytes longirostris*, a new penguin species from Kerguelen Id., collected by the German Deep-sea Expedition in 1899.

This new Mallophagan form unites in striking manner the important antennal characters of the family Philopteridæ with the general habitus and body characters of the family Liotheidæ. The shape of head, and the distinctly free metathoracic segment are characteristics heretofore peculiar to the genera *Menopon* and *Trinoton* (of the Liotheidæ), but the short, slender, five-segmented antennæ not lying in special antennal cavities identify the species as a Philopterid, but one not assignable to any known Philopterid genus. The new form represents a *Menopon*- and *Trinoton*-like genus in that family to which *Menopon* and *Trinoton* do not belong! The only other Mallophagan species taken from the penguin genus *Aptenodytes* is *Goniodes brevipes*, a small species very unlike this new form, described by Giebel (from a female specimen) in the *Phil. Trans. Roy. Soc.*, Vol. 168, extra Vol. This specimen also came from Kerguelen Id.

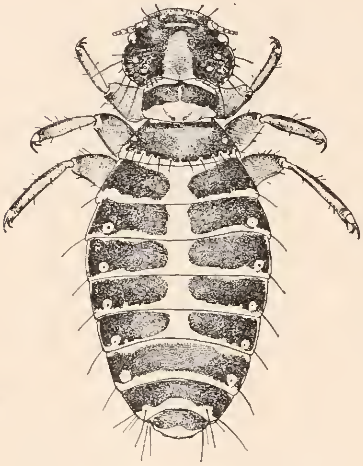

FIG. 3. *Nesiotes demersa*, female. (Length, 5 mm.)

The distinguishing characters of this genus are its *Menopon*-like form, the small suborbicular head with slightly-produced subrectangular temples, the distinctness of the meso- and meta-thoracic segments in a degree unequalled elsewhere among the known Mallophaga unless it be in *Trinoton*, the very small characteristically Philopterid antennæ, the sharp division of each eye into practically a pair of eyes, the large size of the hind body in comparison with the head, the heavy transverse blotches of the abdomen and the five pairs of abdominal spiracles instead of the usual six pairs.

NESIOTINUS DEMERSA sp. nov. (Fig. 3.)

Female. — Body, length 5 mm., width 2.1 mm.; head, length .75 mm., width 1.15 mm.; thorax, length 1.25 mm., width of prothorax .8 mm., width of mesothorax 1.30 mm., width of widest segment, the first, 2.16 mm.; chestnut brown, with large blackish-brown blotches on thorax and abdomen.

Head small in comparison with rest of body, hardly as wide as mesothorax, with flatly rounded front, no orbital sinus, temples slightly swollen, rounded, but with postero-lateral angle slightly obtusely produced, occipital margin slightly curving ; eyes divided so as to give the effect of one pair on each side ; antennæ short, slender, tapering ; pustulated hairs on temporal margins and two small hairs with large pustulation on dorsal surface of each temple, also four smaller pustulations on postero-median dorsal surface, and one mesad from each eye pair ; color chestnut-brown with blackish eye flecks and dark brown markings along temporal margin and in postero-mesial angles of each temporal region.

Thorax of three distinct segments regularly widening posteriorly, the meta-segment being nearly as wide as first (widest) abdominal segment and resembling an abdominal segment ; prothorax with slight median angulated point on anterior margin, with parallel straight lateral margins and rounded antero-lateral and postero-lateral angles, anterior half dark brown, posterior half light brown ; mesothorax with diverging lateral margins, small pustulated hairs in angles and flatly rounding posterior margin : anterior four fifths of segment dark brown with series of weak hairs in demi-pustulations along the hind margin of this dark region ; metathorax with diverging lateral margins, and with large lateral transverse dark brown blotches leaving a rather narrow light brown median space. Legs with heavy short femora and long slender tibiæ with few short, weakly pustulated spiny hairs on each segment ; two terminal tibial spines ; femur darker than the translucent pale brown tibiæ.

Abdomen forming with meso- and metathorax an ellipse ; segments 1 and 2 widest and others tapering slowly posteriorly ; hairs few and inconspicuous ; segments 1–5 with conspicuous spiracles each showing as a small brown spot in a large clear circular pustulation ; segments 1–4 with large lateral transverse dark brown blotches leaving a lighter median space which is narrower on each successive segment posteriorly : segments 5–7 with dark-brown transverse bands extending clear across body ; all transverse blotches and bands blacker and slightly wider at lateral ends, with slight anteriorly projecting process ; indications of demi-pustulations in lateral portions of posterior margin of each blotch and band ; posterior margin of terminal segment flatly rounded, and longest hairs of the body in lateral angles.

NOTE. — In a paper published while this paper was in press, on the Mallophaga from Birds of Costa Rica (Univ. Studies, Vol. 3, pp. 123–197, 1903, Univ. Nebraska) M. A. Carriker, Jr., describes two new genera of Mallophaga, under the names *Ornicholax* and *Kelloggia*.

EXPERIMENTAL STUDIES ON THE DEVELOPMENT OF THE ORGANS IN THE EMBRYO OF THE FOWL (GALLUS DOMESTICUS).

FRANK R. LILLIE.

I. INTRODUCTION.

The results to be described under the above title relate to the morphology, functions and power of regeneration of various embryonic organs, and to the influence that certain embryonic parts exert on the development of others. They represent the application of a particular experimental method, viz., the destruction of definite parts, and study of the subsequent development. Thus the particular organs studied are those most accessible to operation, which form a rather heterogeneous assemblage. Nevertheless, taken as a whole, the results form a contribution to the subject of *correlative differentiation* of organs.

The Principle of Correlative Differentiation in Embryology (*i. e.*, influence of the intraorganic environment in development)[1] is that the rate, degree or mode of differentiation of any embryonic rudiment is dependent on some part or parts of the same organism (individual) external to itself; that is, that component parts of an embryo determine mutually to a greater or lesser extent, their respective lines and grades of differentiation. Much more is meant by this than that any embryonic part can develop only in its normal environment, which offers the prerequisites of its very existence. The principle of correlative differentiation in fact implies a distinction between *a determinative and a non-determinative environment*, and the problem of correlative differentiation is so far resolved when this is ascertained for all the organs (cf. Roux).

Any part, the entire environment of which is non-determinative, is said to develop by *self-differentiation* (Roux).

These two principles do not stand in the relation of rival theories but rather, probably, of coöperative factors in every embryonal differentiation, for any process of self-differentiation of a structure might be analyzable into correlative differentiation of its parts.

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[1] Environment may be defined as conditions that influence dynamic processes in protoplasm, and may be divided into extraorganic and intraorganic, the former being external to the individual and the latter within its bounding surfaces.

For the development of the higher animals at least the extra-organic environment is non-determinative. The development of the ovum as a whole is therefore a process of self-differentiation. But it is usually assumed that it is otherwise with the differentiation of its constituent parts; the extreme view being that each influences the mode of differentiation of all the remainder. From this standpoint the complexity of the correlative processes of differentiation must increase in proportion to the increase in complexity of structure.

Theoretically, at least, the determinative value of correlative differentiation in any case may be (1) absolute, *i. e.*, the mode of development of a part being determined entirely from without itself; (2) partial; (3) wanting, *i. e.*, absolute self-differentiation.

Our present knowledge is enough to exclude the first theoretical possibility. No principle in embryology is better established than that sooner or later the embryo is a mosaic of embryonic rudiments, each of which is to a certain extent self-determining. This mosaic of rudiments may become visible very early, as in those ova exhibiting a definite cell-lineage of organs, or it may appear later. In some cases, at least, the unsegmented ovum itself is a simple mosaic (ovum of ctenophores according to Fischel; ovum of *Unio*, Lillie; ovum of sea-urchins, Boveri; ovum of frog, Roux, Schulze and others). Indeed it is quite probable that all ova are more or less simple mosaics of embryonic rudiments.

Unless, therefore, we wish to beg the entire question we must proceed on the second hypothesis. This is the writer's standpoint, and the problem is to determine as many definite correlations as possible and to investigate their nature.

There is probably no conception in embryology so vague as that of correlative differentiation, as the following citations may serve to show:

Hertwig: "Zelle und Gewebe," II.:

"Die Wechselwirkungen (Correlationen) zwischen den Zellen eines Organismus und ihren Derivaten bilden sich mit dem

Beginn des Entwicklungsprocesses aus, ändern sich von Stufe zu Stufe und compliciren sich in demselben Maasse, als die Entwicklung fortschreitet.

"Im Gegensatz zum Mosaiktheorie von Roux und der keimplasma theorie von Weismann stellt die Theorie der Biogenesis den Grundsatz auf, dass vom ersten Beginn der Entwicklung an die durch Theilung des Eies sich bildenden Zellen beständig in engster Beziehung zu einander stehen, und dass dadurch die Gestaltung des Entwicklungsprocess sehr wesentich mit bestimmt wird. *Die Zellen determiniren sich zu ihrer späteren Eigenart nicht selbst, sondern werden nach Gesetzen die sich aus dem Zusammenwirkung aller Zellen auf den jeweiligen Entwicklungsstufen des Gesammtorganismus ergeben, determinirt.*"

Herbst: "Formative Reize in der Tierischen Ontogenese:"

"Die Aufgabe des zweiten Teiles meiner Abhandlung über die formativen Reize war es also, in der tierischen Ontogenese, abgesehen von der Namhaftmachung jener wenigen Fälle von Gekommen von formativen Reizwirkungen, die von irgend einem Teil des Organismus auf einen oder mehrere andere ausgeübt werden, festzustellen und eventuell die Möglichkeit der vollständigen Auflösung der ganzen Ontogenese in einer Reihe von solchen Induktionserscheinungen nachzuweisen.

"So ist es zum Beispiel zum mindesten ungenau, von der 'weitgehenden Wechselbeziehung' zo sprechen, 'die zwischen allen Teilen eines Organismus auf allen Stadien seiner Entwicklung besteht' (Hertwig: 'Evolution und Epigenesis'); denn das Ektoderm der Echiniden entwickelt sich unabhängig vom Entoderm, und auch abgeschnürte Hautstücke, etc., konnen sich selbständig differenzieren, wie dies das Vorkommen der Teratome beweist (Roux). Die Annahme einer ganz allgemeinen Korrelation zwischen allen Teilen des Organismus auf allen Stadien der Ontogenese ist deshalb ebenso falsch wie jene von der qualitativ ungleichen Kernteilung der Mosaiktheorie."

Most of the real illustrations (*i. e.*, experimentally determined) of this principle must be taken from plants and plant-like animal colonies. One need only glance through Herbst's recent "Formative Reize in der Tierischen Ontogenese" to realize that, so far as egg development is concerned, the application of the prin-

ciple rests very largely on inference, analogy and a few doubtful pathological conditions.

Discussion of this subject belongs, however, to the conclusion rather than to the introduction, and the foregoing remarks are intended only to define the problem.

II. Methods of Operation.

In making the operations one must work as far as possible under antiseptic conditions. Instruments, etc., must be sterilized; this is most readily done by passing the needles, knives, scissors, etc., through a flame immediately before each is used. In spite of all precaution a great many eggs are infected. In my experiments only about 20 per cent. of the eggs remained alive until the time of examination for the results of the experiment, two to five days after the operation. The causes of the mortality in the remaining 80 per cent. are two: (1) Fatal injury of the operation (about 40 per cent. ?); (2) infection with mould or bacteria (about 40 per cent. ?). There is a very noticeable difference between different lots of eggs; some bear operations much more readily than others and are less prone to infection. These differences in the relative powers of resistance of different lots of eggs are due to the relative freshness of the eggs when incubation is begun, and also to the time of year. It is noticeable that in a lot of eggs in which a relatively large proportion, over 50 per cent., fail to develop in the incubator, the percentage of failures in the actual experiments is usually very high.

The method of procedure in my experiments was as follows:

1. The eggs are not turned in the incubator, so that one may be sure of locating the position of the embryo in the unopened egg exactly. The upper side of each egg is marked with a pencil.

2. A small opening is made through the shell and membrane over the embryo.

3. The operation is then made. For cauterization I employ either a needle heated red hot in the flame, or an electric cauterizing needle. The heated needle cools very rapidly, so that the operation must be hastily performed, and it is difficult precisely to delimit the injury. The electric cautery, on the other hand, is apt to give too intense heat. Each method possesses certain advantages.

4. The opening in the egg is closed as follows: A piece of the shell with membrane attached is cut from a corresponding part of another fresh egg, so as to be slightly larger than the opening in the operated egg. This is placed over the opening so as to close it completely; and the albumen adhering to the membrane acts as cement. To ensure perfect closure strips of the egg-membrane are plastered on so as to overlap all edges of the foreign shell. The advantages of this method of closure are that the foreign surfaces are perfectly aseptic if fresh eggs are used, and that the conditions are as nearly like the normal as possible. It is, morover, the simplest and easiest method. This method of closing the opening was first used by Miss Peebles.[1]

III. Experiments on the Amnion and the Production of Anamniote Embryos in the Chick.

A. *The Normal Development of the Amnion.*

The purpose of this section is to give a brief statement of some facts concerning the formation of the amnion before taking up the analysis of the processes by experiment. This is necessary because the facts are at least partly new, and without knowledge of them the mechanics of formation of the amnion cannot be understood. For a recent review of the literature on the whole subject of the amnion in the Sauropsida, see Schauinsland ('02*a* and '02*b*); the latter paper I regret not to have seen.

In the somatopleure on each side of the axis of an early embryo of the chick three zones may be distinguished on the basis of the subsequent differentiation, (*A*) for the body-wall; (*B*) for the amnion; (*C*) for the chorion (serosa) (Fig. 1). It is important to trace the origin of the differentiation between the amnion and serosa on the one hand, and amnion and body-wall on the other, for the conditions that determine the development of the amnion must be antecedent to such differentiation.

1. *The Ectamnion.*—The differentiation of the amniogenous from the choriogenous somatopleure is always preceded by the appearance of a thickening of the ectoderm along the external margin of the former. This thickening, for which I propose the

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[1] *Roux's Archiv*, VII., 1898.

name ectamnion, precedes by a little the formation of amniogenous folds in any region, and indeed it induces the origin of the entire system of folds. It has been described by many embryologists at the stages immediately preceding fusion of the limbs of the amnion (cf. Schenk, '71), and it forms the ectodermal sero-amniotic connection of Hirota ('94). But no one, so far as I know, has traced it back to its origin and recognized the fact that it is the earliest formed part of the amnion, which is thus primarily ectodermal in the chick, as in Chelonia and some other primitive Sauropsida.

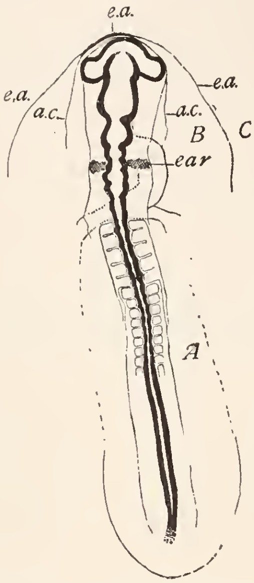

FIG. 1. Embryo of chick with 13 mesoblastic somites. University of Chicago Embryological Collection, No. 555. *e.a.*, ectamnion; *a.c.*, inner margin of amniocardiac vesicles; *A*, region of the somatopleure destined to form the body-wall; *B*, amniogenous somatopleure; *C*, choriogenous somatopleure.

The ectamnion may first be distinguished at about the stage with nine mesoblastic somities, where it appears as a median thickening of the ectoderm in front of the head near the anterior boundary of the proamnion. Along the line of this thickening there is a fusion, between ectoderm and entoderm. The thickening is extended right and left and turns backwards along opposite sides of the héad to about the region of the middle of the heart, gradually becoming more peripheral in position and slowly fading out (Fig. 1). This line represents the junction of the amniogenous and choriogenous somatopleure, and thus corresponds to the angles of the future amniotic folds.

The head of the embryo lies in a depression bounded in front by the ectamnion and on the sides by the amnio-cardiac vesicles of the body cavity, along the inner upper margin of which the ectamnion runs for a short distance. The floor of the depression is the proamnion.

In a stage with 14–15 mesoblastic somites the ectoderm of the proamnion is much more thickened in front of the head, and has a *villous outer surface* in consequence of irregularity in the thickening[1] (Fig. 2), which may be traced back to the level of the heart, and on one side to its hinder end; there is also a very short ectentodermal fusion beneath the tip of the head. In this series the ectamnion marks the boundary between two distinctly differentiated parts of the extraembryonic somatopleure, the more central of which is the amnion.

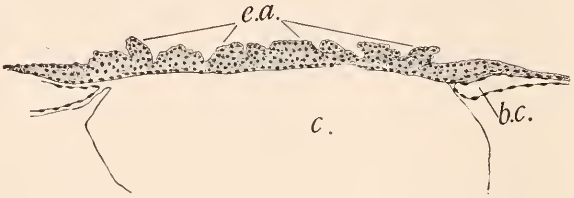

FIG. 2. Transverse section through the anterior angle of the ectamnion, a few sections in front of the tip of the head. 14–15 mesoblastic somites. University of Chicago Embryological Collection, No. 215. *b.c.*, body-cavity; *c.*, large cavity in the entoderm; *e.a.*, ectamnion.

In another embryo with fourteen mesoblastic somites, the tip of the head is surrounded by the amnion, and the proamniotic part is represented only by a short median strip extending eight sections back to a point where the limbs of the amnion have not yet closed. The ectamnion is continued only for a short distance along the *angles* of the amniotic fold, and then passes peripherally. How has the head-fold been formed ? The great expansion of the body cavity (amniocardiac vesicles) on each side causes an elevation of the anterior angle of the ectamnion and a pocket is formed by fusion of its opposite limbs, which have a strong affinity for each other ; fusion proceeds along the median dorsal line so long as the energy of fusion is sufficient to draw the somatopleure up. The head of the embryo is rapidly elongating at this time and slips into the pocket thus formed, being guided in part by the cranial flexure (Fig. 1). It is interesting to note how far the ectodermal thickening stretches ahead of the mesoderm of the fold near the point of closure, and that the apical cells are elongated into pseudopodium-like processes.

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[1] In examining the section one receives a strong impression that the irregularities may be due to amœboid movements; but it is not possible to confirm this by actual observations.

The histological differentiation of the amniotic area of the somatopleure from the chorionic portion precedes the elevation of the fold.

This brief inquiry, then, suggests that the order of events in the formation of the head fold of the amnion is :

1. Thickening of the ectoderm on the outer margin of the amniogenous somatopleure, beginning in front of the head of the embryo and extending back on each side (ectamnion).

2. Great expansion of the body cavity on each side opposite the head of the embryo and consequent elevation of the anterior bay of the ectamnion to the level of the dorsal surface of the embryo.

3. Fusion of the right and left limbs of the ectamnion, beginning at the angle, to form a pocket, the head-fold of the amnion.

4. Pushing of the head of the embryo into the fold.

There may be, however, considerable variation in the time of formation of the head-fold. I have, for instance, one series with 17–18 mesoblastic somites (ser. 175), where the head-fold is not yet formed.

Extension of the Ectamnion. — The ectamnion differentiates backward more rapidly than the lateral folds, and always precedes their origin. In the 48-hour stage (21–22 somites) (Fig. 3) the ectamnion from in front has joined that from behind formed in connection with the tail-fold. There is a place, corresponding nearly to the final meeting place of anterior and posterior lateral folds, where it becomes very faint. It would appear then that behind the tail there is actually a new starting-point for the ectamnion as well as the amniotic folds. The primary position of the ectamnion is near the boundary of the pellucid area ; towards the posterior end it bends in very sharply, nearly joining the body wall proper, and terminating in the posterior rudiment.

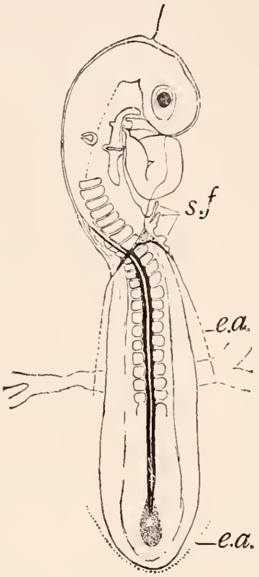

FIG. 3. Embryo of chick with 21 mesoblastic somites. University of Chicago Embryological Collection, No. 99. *e.a.*, ectamnion ; *s.f.*, secondary folds of the amnion on the right side. The dotted line continuing *e.a.* represents the continuation of the ectamnion beyond the region of folding. The dotted area at the angle of the folds represents the ectodermal sero-amniotic connection of Hirota.

Origin of the Tail-Fold. — The tail-fold proper arises from an ectodermal thickening lying in a depression just beneath the rudimentary tail-bud. The depression is caused by the enlargement of the body cavity on each side of the middle line. These enlargements may be called the amnio-allantoic enlargements, as they are associated with the formation of the allantois. I would venture the hypothesis that the existence of a *separate* tail-fold of the amnion is associated with the time of development of the allantois, which is represented in the embryo under consideration (1) by a shallow entodermal evagination and (2) a mass of mesoblast.

At the time of formation of the tail-bud a very shallow pocket forms behind it. This owes its origin to the elevation of lateral folds of the somatopleure and progressive fusion beginning at the posterior angle of the ectamnion. The floor of the pocket includes a thick posterior prolongation of the allantoic mesoblast which furnishes a firm floor to the pocket and thus determines the form of the folds.

2. *The Amniotic Folds.* — The subsequent development includes the elevation and fusion of the anterior and posterior lateral folds. The final closure takes place opposite the buds of the hind limbs. The order of events in these processes is as follows:

1. The growth of the amniogenous somatopleure behind the head-fold and in front of the tail-fold.
2. The uprising of the amniotic folds, and their growth in a definite direction around the embryo.
3. The fusion of the right and left folds along the line of the ectamnion in such a way that the external limbs unite to form the chorion, and the internal to form the amnion.

Study of the morphology of these processes suggests the following physiological conclusions:

1. The growth of the amniogenous somatopleure may be a result of the traction exerted in it by the progressive fusion of the folds already formed in front and behind.
2. The uprising of the lateral folds is determined by the head- and tail-folds, the progressive fusion of the right and left ectamnion dragging the amniogenous somatopleure into place.

It remains to test these conclusions by experiments, but before proceeding to a description of these, I wish to describe *the influence of the rotation of the embryo on the amniogenous somatopleure.*

Practically all of the somatopleure of the pellucid area is amniogenous with the exception, naturally, of that part internal to the limiting sulci that forms the body-wall. What effect has the turning of the embryo on its left side on the amniogenous somatopleure? We will suppose that the latter is primitively of equal width on both sides; we will furthermore assume that the somatopleure cannot be drawn in from the vascular area, because it is here attached to the splanchnopleure. (The fusion of the somatopleure and splanchnopleure at the margin of the pellucid area is shown by the fact that the splanchnopleure is often drawn up with the outer limb of the amniotic fold, making a fold of the splanchnopleure at this place) (Fig. 5). Finally let us assume that the notochord represents approximately the axis of rotation. During the process of rotation the embryo sinks and the lateral limiting sulci become deeper. A direct consequence of the rotation must be therefore a strong tension on the somatopleure belonging to the under (left) side, *a-b*, and practically none on the upper (right) side, *c-d*, (see Fig. 4, *A*, *B*, *C*).

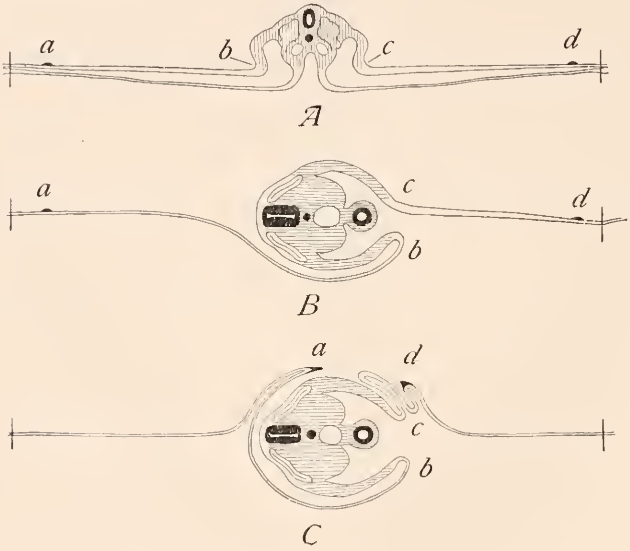

Fig. 4. *A*, *B* and *C*. Diagrams to represent the effect of rotation of the embryo on the amniogenous somatopleure. *a* represents in all figures the position of the ectamnion on the left (lower) side; *d* represents in all figures the position of the ectamnion on the right (upper) side. *b* and *c* represent the junction of amnion and body-wall on left and right sides respectively. In Fig. *A*, *a–b* and *c–d* are equal. In Fig. *B*, rotation of the embryo is assumed to have taken place without formation of the amnion; the distance *a–b* has become greater than *c–d*. In Fig. *C* is represented rotation of the embryo with synchronous formation of the amniotic folds, as is actually the case; *c–d* is inevitably thrown into secondary folds. The vertical lines at the extreme right and left represent the margins of the pellucid area.

Even though the difference may be partly compensated for by drawing of the embryo to the left, the tendency would be to stretch *a-b*. If there were no such compensation and *a* and *b* were practically fixed points, the length of *a-b* at the conclusion of the rotation would much exceed that of *c-d* (Fig. 4, *b*); and if during this process there were actual independent growth of *a-b* and *c-d*, the latter would of necessity be thrown into folds, but not the former. Finally, if the amniotic folds were forming at the same time (as is actually the case) the right one would inevitably be thrown into secondary folds by the approximation of points *c* and *d* (Fig. 4, *C*).

Study of the fusion of the amniotic folds in actual section shows (1) that the line of fusion of the opposite amniotic limbs is over the dorsal surface of the embryo *only so long as the latter lies flat on the yolk*, and does not follow the turning of the embryo on to (usually) its left side; the consequence is that after rotation of the embryo the line of fusion lies over the upper (right) side of the embryo, often opposite the horizontal level of the intestine (Fig. 6). Thus one fold of the amnion passes all the way from the under side over the back of the embryo and around on the other side to the line of fusion, and thus is several times as long as the opposite limb. (2) Moreover, the amniotic fold of the right side is invariably thicker than that of the left side, and is always thrown into secondary folds at the place of turning (Fig. 5 and Fig. 6). These conditions are satisfactorily explained, as noted above, by the mere turning of the embryo on its side.

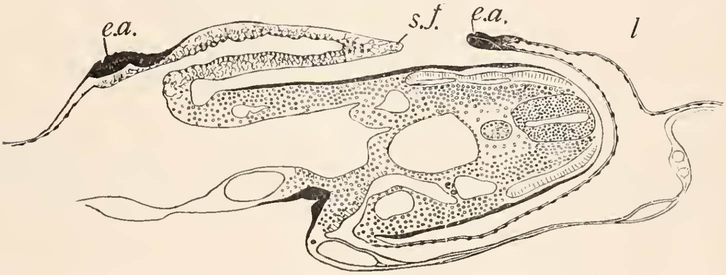

FIG. 5. Transverse section of an embryo of about 48 hours (Duval) showing the position of the ectamnion on the right and left sides. University of Chicago Embryological Collection, No. 689. *e.a.*, ectamnion; *l.*, left; *s.f.*, secondary fold of amnion on the right side. The great differences in the thickness of the amnion of the right and left sides should be noted.

One must therefore distinguish in the upper limb of the amnion two kinds of folds: (1) The ordinary amniotic fold induced by the fusion of the right and left rudiments and (2) *secondary* *folds* formed simply by the process of twisting of the embryo. This distinction is of importance in interpreting the results of the experiments.

Hirota (94) notices the secondary fold on the upper side and says: "It seems to owe its origin to the presence of the sero-amniotic connection. . . . It is always on the right side of the connection, and is pushed on towards the left. There takes place no folding before the allantois appears, and the longitudinal extent of the fold depends on the extent of the sero-amniotic connection." "Its form and extent are variable." "It is not clear what significance this fold has." "At both extremities of the sero-amniotic connection the amnion is also slightly folded longitudinally."

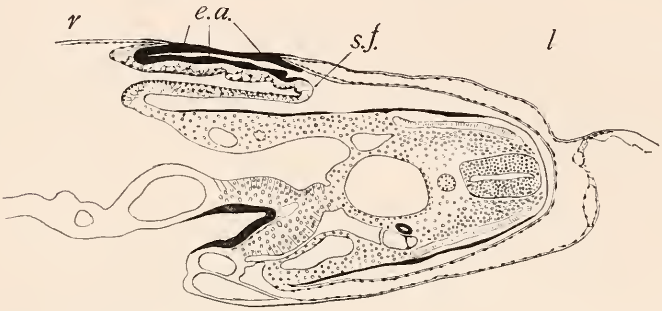

FIG. 6. Section of the same embryo as the preceding, 10 sections (150μ) in front of Fig. 5. The section passes through the place of fusion of the right and left folds. The secondary fold of the amnion is well shown on the right side. Letters as in Fig. 5.

These secondary folds of the amnion are very transitory except in two regions: (1) Above the hind end of the heart (apex of ventricle) and continuing a short distance behind it; (2) in the region immediately in front of the allantois, at 60–70 hours, thus in the neighborhood of the final closure of the amniotic folds. The former are of very constant occurrence and persist a long time (Fig. 3). The latter are relatively slight and inconstant. Hirota is thus mistaken in saying that these folds do not appear until the formation of the allantois.

The secondary folds in the neighborhood of the heart are always on the upper (right) side; they first appear at the time of rotation of the embryo, and are coincident with the closure of the amnion (Fig. 3); they persist until the body-wall is completed behind the entire heart. They are not, in my opinion, exclusively folds of the amnion, but extensions of the body-wall for enclosure of the region of the heart and liver. The direct cause of their formation is, however, the rotation of the embryo with extreme growth of the body-wall contiguous to the amnion, and fixation of the outer end of this limb of the amnion by the amniotic suture.

Elsewhere the effect of the twisting of the embryo is rapidly compensated so that the secondary folds of the right half of the amnion do not persist long except in the region of the allantois, where slight inconstant secondary folds may continue longer.

B. *Experimental.*

1. *Experiments on the Head-fold of the Amnion.*

Experiment No. 57.

Age of the embryo at the time of operation, 33 hours[1] (Duval).

Operation.—The blastoderm was cauterized lateral to the right optic vesicle with a needle (Fig. 7) so as to make a large opening. At the time of the operation only the most anterior horseshoe-shaped segment of the ectamnion was present (cf. Fig. 1), and this was destroyed only on the right side of the embryo. On the left side, therefore, the amniotic fold was free to form to the extent that it is independent of the opposite fold. The right optic vesicle was slightly injured, as the results of the experiments show. In opening the egg for the operation, the blastoderm was also inadvertently torn just back of the embryo, and this opening also appears in Figs. 8 and 9. This, however, was without any noticeable effect on the subsequent development.

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[1] In describing the various experiments, the age of the embryo at the time of the operation will not be given as the actual number of hours in the incubator, because the variations in point of actual development after the same period of incubation are so extreme. It is not possible either to make accurate measurements of the living embryo or to determine the number of somites present, on account of the loss of time and danger of exposure of the embryo. A rough sketch of the embryo was always made at the time of the operation, and this is sufficient to identify it with the various stages figured in Duval's atlas. The age is based on this identification. Thus the given age at the time of operation in these experiments represents a certain definite stage of development. On the other hand, the length of time that elapsed from the experiment to the time of reopening the egg is always given literally.

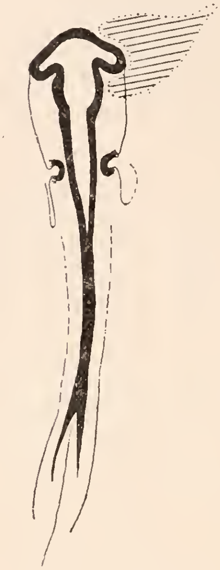

FIG. 7. Experiment 57. Operation diagram. Outline of embryo of chick of about 33 hours, after Duval. The ruled area to the right of the head indicates the area of the blastoderm destroyed by the heated needle.

Examination of the Resulting Embryo.—The egg was reopened 48 hours after the operation. The heart was beating vigorously; the hole made in the blastoderm by the operation had not closed, and a good deal of yolk had escaped through this and overlay the blastoderm. The embryo was well developed, corresponding to the stage of 70–80 hours (Duval), and apparently normal in all essential respects. (A defect in the right eye was evidently a direct result of the operation.) The head of the embryo had slipped through the hole in the blastoderm and was suspended in the yolk (Figs. 8 and 9).

The embryo was cut into 250 sections of 15 μ thickness. Around the edges of the opening made by the operation the somatopleure turns over and becomes continuous with the splanchnopleure, ectoderm with entoderm, and mesoderm with mesoderm. In places one cannot determine where the ectoderm leaves off and the entoderm begins.

Amniotic Rudiments of the Left Side. — A short distance in front of the margin of the opening there is a sharply defined fold of the somatopleure capped by an ectodermal thickening that represents the head-fold and left lateral fold of the amnion. The extent of this fold is indicated by the line *e.a.* on Fig. 8. It begins as a sharply marked fold at the most anterior angle of the opening, and passes back, at first along the edge of the opening, later a short distance from it, to the left of the embryo. It very distinct (Fig. 10, *l.a.f.*) to the point where it is indicated as is a broken line; in this region the fold has disappeared, but the thickening of the ectoderm (ectamnion), may be traced back to the tail-fold with which it becomes continuous as indicated in the drawing (Fig. 8). At no place, until the tail-fold is reached, is the somatopleure internal to this line thrown into folds. By

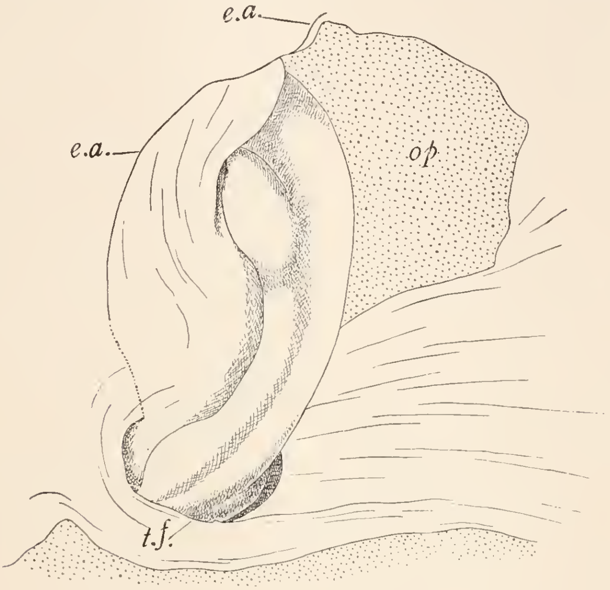

FIG. 8. Experiment 57. Upper surface of blastoderm. *op.*, aperture in the blastoderm made by the operation; *e.a.*, amniotic rudiment of the left side; *t.f.*, tail-fold of the amnion. The stippled area behind the embryo represents an aperture in the blastoderm accidentally made in opening the egg for the operation.

reference to the figure and to the description of the operation it will be seen that the line of this fold represents the continuation of the left amniotic rudiment, which was not injured by the operation.

I conclude, therefore, that when the amniotic rudiment of one side is left free to develop after destruction of the rudiment of the other side just prior to the formation of the head fold, the ectamnion is propagated in the normal fashion and induces the formation of a low fold, but that the amniogenous somatopleure is unable to raise itself around the body of the embryo. The growth of the amniogenous somatopleure appears to be less than normal.

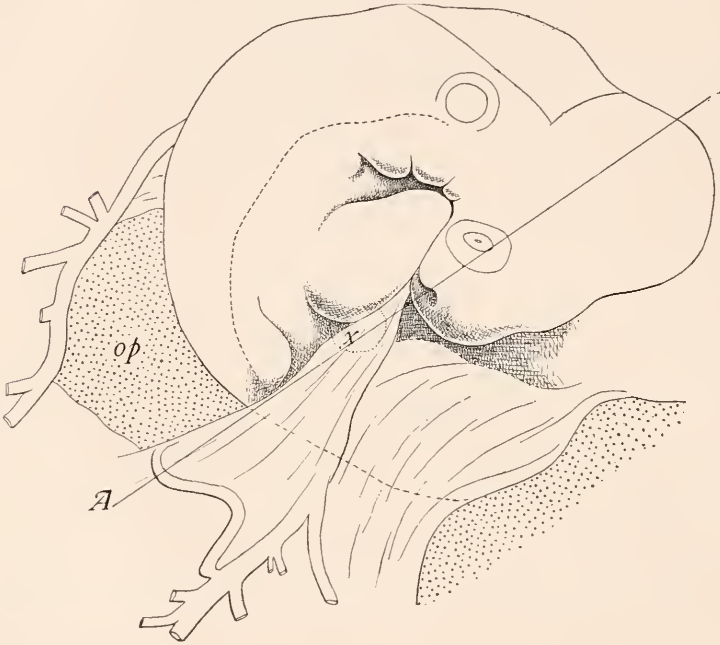

FIG. 9. Experiment 57. Under surface of the blastoderm. There is no amnion. The right eye is defective. *x* marks the location of the secondary amniotic fold shown in Fig. 10. *A–B*, plane of section shown in Fig. 10. Letters as in Fig. 8.

Amniotic Rudiments of the Right Side. — On the right side, on the other hand, a well-developed fold appears at the place where the extra-embryonic somatopleure becomes continuous with the body-wall (section 138, Fig. 10) and extends to section 167, where it suddenly ceases, a distance of about 0.5 mm. The location is indicated by *x* on Fig. 9, and Fig. 10 shows it in section.

The formation of this fold is not induced by the ectamnion because the line of the latter (Fig. 10, *r.e.a.*) may be recognized some distance lateral to the fold, through it is very slightly developed. The fold in question is immediately back of the heart on the right side of the body. It is not, in my opinion, a true amniotic fold, but belongs to the category of normal secondary folds of the amniogenous somatopleure produced by the turning of the embryo, with which it agrees precisely in position and appearance. This conclusion is reinforced by the following consideration : in this embryo the roots of the vitelline veins are prolonged forward to an abnormal extent, and the right vein is fused to the somatopleure lateral to the fold (Fig. 10). As the embryo turns, therefore, the somatopleure between the vitelline vein and the body-wall must be folded to the extent that the turning approximates the body-wall to the vein, because the fusion prevents the somatopleure from being pushed peripherally. As already said, therefore, this is not a true amniotic fold.

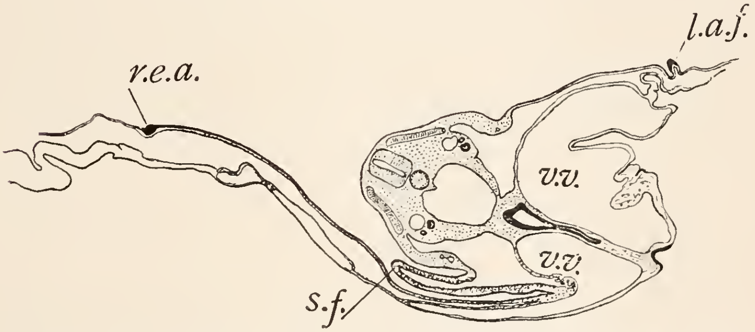

FIG. 10. Section through the embryo of experiment 57 along the line *A–B* of Fig. 9. *l.a.f.*, left amniotic rudiment ; *r.e.a.*, ectamnion of the right side ; *s.f.*, secondary fold of amnion on the right side ; *v.v.*, vitelline veins.

The prevention of the formation of the head-fold, by destruction of the rudiment of one side, operates to prevent the normal elevation of the amniotic fold on the opposite side ; and thus it is experimentally demonstrated that the coöperation of right and left folds is necessary for the normal mode and direction of growth of the amniotic rudiments. The height of the fold on the uninjured side is a measure of the power of independent elevation of a single amniotic fold.

On the other hand the existence of the ectamnion on the right side, though in a rudimentary state, and the differences in finer structure of the somatopleure on the two sides of this line indicate that the distinction between amniogenous and choriogenous somatopleure is attained by the normal development of the somatopleure as a whole, and not simply as a result of their separation after fusion. However, the relatively rudimentary condition of the ectamnion on the injured side shows that the earlier stimulate the growth of the latter formed parts ; otherwise we should expect to find the ectamnion equally developed on both sides. The ectamnion of the right side does not exactly join the tail-fold.

Tail-fold. — The tail-fold of the amnion may be well seen in Fig. 8. So far from compensating in any way for the absence of head and lateral folds, it is of even less than its normal extent, a fact indicating (possibly) that normally its growth is stimulated by the traction of the anterior section of the amnion.

Experiment No. 36.

Age of the embryo at the time of operation forty-six hours (Duval).

Operation. — The operation consisted in the insertion of a heated needle just in front of the heart (see Fig. 11). Examination of the sections of the resulting embryo shows that the injury involved the left optic cup slightly, and that the head-fold of the amnion which extends back beyond the heart at this stage, stuck to the needle and was stripped off, carrying with it a certain amount of the adjacent somatopleure. This was not observed at the time of the operation, but the conclusion is rendered positive by the subsequent examination of the embryo.

Examination of the Resulting Embryo.

The egg was reopened and the embryo preserved forty-eight hours after the operation. The embryo (Fig. 12) appeared like a normal embryo of about the ninety-sixth hour. The limb-buds were well started, and the allantois extended out beyond the embryo, but towards the dorsal surface; the flexures were normal. The striking thing was the apparent entire absence of the amnion; the embryo lay naked on the surface of the blastoderm, to which it was attached, in the same manner as a selachian embryo by a very broad somatic and splanchnic umbilicus.

In the normal embryo of this age the amnion is completely closed, and the body-wall of the embryo has, therefore, lost all connection with the chorion.

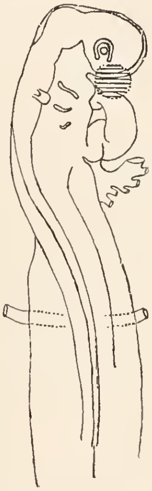

FIG. 11. Experiment 36. Operation diagram. Outline of embryo of chick of about 46 hours, after Duval. The ruled area shows the site of the operation with the heated needle. For description of the operation see text.

This embryo was cut into 625 transverse sections. These confirm the general absence of the amnion, and at the same time furnish additional data. Back to about the 354th section (forty sections behind the heart), the somatopleure beneath the embryo is entirely missing; evidently it had been torn away by the operation and had not been replaced. Throughout this region the extra-embryonic somatopleure begins on each side of the embryo with a free edge. A short distance behind the heart, folded portions of the original amnion appear lying in the gap in the somatopleure, and continuous with the midventral line of the body-wall. Beginning with about the 371st section (see Fig. 13) the body wall is open ventrally, and is continuous with the extra-embryonic somatopleure on one side, while on the other the original gap in the somatopleure is still open (see Fig. 13). In this region, the somatopleure for some distance external to

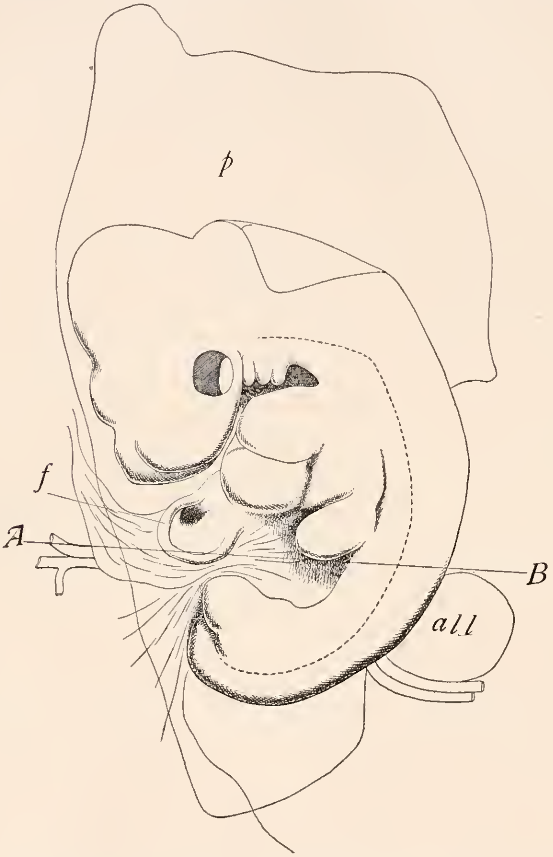

FIG. 12. Experiment 36. Surface view of embryo; upper surface of blastoderm. The embryo is anamniote, except for a rudimentary tail-fold. *all.*, allantois; *p.*, pellucid area. *A–B*, plane of section shown in Fig. 13; *f.*, fold of somatopleure.

the part destined for the body-wall is thrown on both sides into irregular folds that obviously represent the lateral amniotic folds. They rapidly decrease in size posteriorly, and *almost* completely disappear in the region extending from the 420th section back, *i. e.*, a short distance back of the fore-limbs. Beginning opposite the hind-limbs the folds again increase in size. They are very irregular and do not form the normal investment of the tail. But beneath the latter they form a closed pocket, the usual tail-fold.

Over the entire region, extending from about the posterior edge of the fore-limb to the beginning of the hind-limb, there are no folds in the amniogenous somatopleure. This would indicate that the normal rapid growth of this region is progressively induced under normal conditions by the extension of the lateral angles of the head-fold backwards. The folds shown in the figure are only from about 354–430 and may be explained as remnants of the original head-fold, the postero-lateral prolongations of which were probably not entirely removed by the operation. These folds have not, however, united over the embryo nor have they induced formation of folds behind them. The reason for this is clear when we consider that the normal process involves continuous traction on the somatopleure back of the advancing folds, for the latter are continually fusing along the dorsal line with those of the opposite side and thus are constantly, so to speak, gathering in the slack, and causing tension.

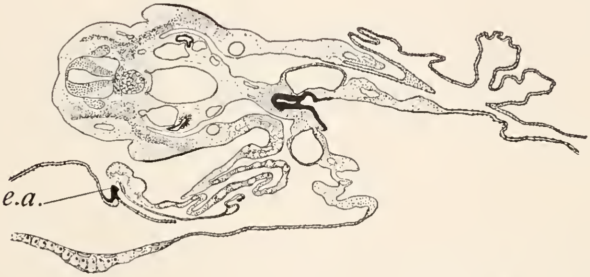

FIG. 13. Experiment 36. Section of embryo along the line *A–B*, Fig. 12. The irregular and incomplete amniotic folds are well shown. *e.a.*, ectamnion of the right side.

In the drawing of the entire embryo, the left side is uppermost, but at the time of the operation the right side was up. Evidently the embryo was turned over after removal of the blastoderm in the process of preparation. This explains why in the section the lower amniotic fold has the usual appearance of the upper fold. The ectamnion is visible only on the left side of the drawing; on the right side no trace of it could be found, except in the region of the tail-fold.

The results of the destruction of the head-fold of the amnion in the stage of 46 hours are: (1) Inhibition of the progressive differentiation of the amniotic zone of the somatopleure; (2) failure of the parts of the lateral folds left to unite around the embryo. The failure of the amniotic folds to unite in the region where they are best formed and are of more than sufficient length for enclosure of the embryo shows that the normal union of the folds is due to the guidance and support of the earlier formed parts of the amnion.

The tail-fold, however, forms in a fairly normal manner. The actual abnormalities in this fold are probably secondary, that is, probably due not so much to direct disturbance of the amnion itself as to the freedom of movement of the embryo permitted by the absence of the head-fold, resulting in the withdrawal of the tail of the embryo from the forming tail-fold.

The body-wall is unenclosed for 113 sections; in a normal embryo of about the same age the body-wall is unenclosed for about 55 sections. Thus it would appear that the closure has been delayed.

Experiment 60.

Age of the embryo at the time of operation about 33 hours.

Operation.—The blastoderm was cauterized just lateral to the right optic vesicle, as in experiment 57, producing a large opening (Fig. 14).

The egg was reopened 72 hours after the operation, and a large, finely developed vascular area was seen with apparently no embryo. But more careful examination revealed the naked hind quarters of an embryo sticking up near the center of the vascular area, the whole trunk and head of which were plunged through the blastoderm into the yolk. The head and trunk of the embryo had slipped through the hole made by the operation into the yolk-sac, and the edges of the blastoderm around the original opening had fused in such a way as to close around the hinder part of the embryo. A large part of the vascular area was cut out and the embryo was gently floated into a watch crystal of physiological salt solution. Turning over the blastoderm, the embryo was revealed entirely without an amnion (Fig. 15). Not even the tail-fold was found.

The embryo is represented in Fig. 15 as it lies on the reversed blastoderm, the entodermal face of which is up. The allantois is well developed and lies in a special enlargement of the body cavity behind the embryo.

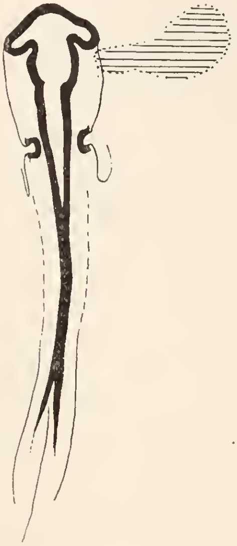

FIG. 14. Experiment 60, operation diagram. Outline of embryo of chick of about 33 hours, after Duval. The ruled area indicates the region of the blastoderm destroyed by the operation.

In this experiment, as in experiment 57, only the right limb of the ectamnion of the prospective head-fold was destroyed; and the consequence of this is in both cases the suppression of the amnion with the exception of the tail-fold. In this case the various membranes have been so confused by the curious position of the embryo and by various secondary fusions that it is quite impossible to determine the behavior of the uninjured rudiment of the amnion of the left side. A single section may serve to illustrate one of the very peculiar conditions (Fig. 16). Lying above the embryo is seen the blastoderm composed of the somatopleure and splanchnopleure. The body-wall of the embryo has fused with the splanchnopleure in such a way that the two are directly continuous on both sides, and the body wall may be traced directly into the wall of the intestine. The result of this fusion must have been an opening on each side into the yolk-sac; but this has been roofed over by extension of the blastoderm surrounding it. Farther back the wall of the intestine becomes continuous with the extra-embryonic splanchnopleure.

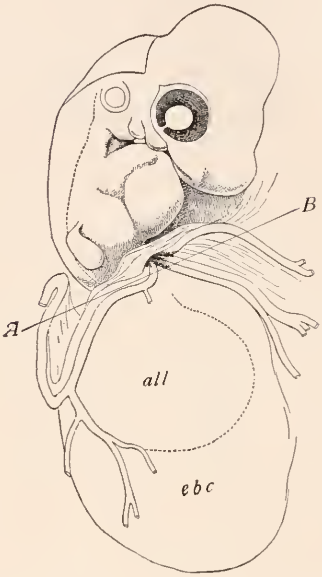

FIG. 15. Experiment 60. Under *i.e.* entodermal, surface of the blastoderm. The embryo is anamniote, but otherwise quite perfect. *all*, allantois; *e.b.c.*, extra-embryonic body-cavity; vitelline arteries and veins shown *A-B*, plane of the section shown in Fig. 16. The embryo was suspended within the yolk-sac, as described in the text.

In the region of the tail rudiments of the tail-fold of the amnion are found.

Two other completely anamniotic embryos (numbers 112 and 124) were produced by experiments similar to those already described. Both of these had passed through the hole made in the blastoderm and were suspended within the yolk-sac. One of these was much farther developed than number 60. They confirm the general results of the dependence of amnion formation on the presence of the head-fold. They possess other definite lesions, the effects of which will be described in another paper.

Condition of the Allantois in Anamniotic Embryos.

The allantois is well formed in four of these embryos; one (No. 57) was too young to show it externally. It is obvious that in the absence of the amnion the growth of the allantois must be attended with difficulties. When the amnion is normally formed a large free space is created above and around it, into which the allantois can freely spread. The absence of this space causes compression of the allantois, and changes the direction of its growth, but I do not think that the latter is much impeded. The mechanical force of the expansion of the allantois causes separation of the somatopleure and splanchnopleure to proceed more rapidly in its immediate vicinity than elsewhere (see Fig. 15). In experiment 124 the greater diameter of the allantois exceeds the greatest length of the embryo. I see no reason why this process might not provide all necessary space for its expansion. It might be, however, that the resistance offered would tend to cause accumulation of the products of excretion in the body of the embryo, and thus gradually poison it.

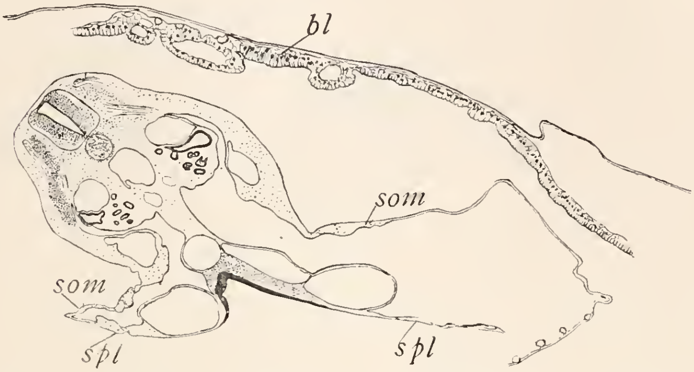

FIG. 16. Experiment 60. Section along the line *A-B*, Fig. 15. *bl.*, blastoderm overlying the embryo; *som*, somatopleure; *spl.*, splanchnopleure. On the right side there is a break in the continuity of somatopleure and splanchnopleure; this was evidently produced in the preparation, as the continuity is perfect some distance in front, and also behind.

2. *Experiments on the Tail-Fold of the Amnion.*

I have also made a number of experiments on destruction of the tail-fold of the amnion. The results are in most cases complicated by conditions that do not properly belong to the subject of this paper. There is but one uncomplicated case (exp. 18). In this experiment the hind-end of the embryo was cauterized immediately after the appearance of the tail-bud (Fig. 17), thus destroying the tail-fold of the amnion. When the egg was reopened forty-eight hours later, a well-developed embryo of about five days was found in which the amnion ceased with a free edge immediately in front of the hind-limbs (Fig. 18).

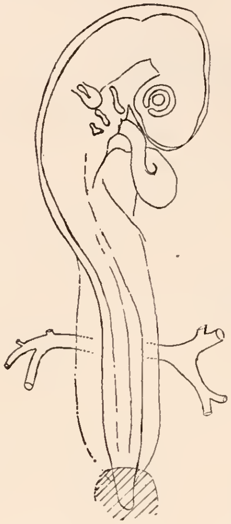

FIG. 17. Experiment 18, operation diagram. Outline of embryo of chick of about 52 hours, after Duval. The ruled area represents the part destroyed by the heated needle.

The conditions of the membranes in this embryo are otherwise very complicated and difficult to understand. Thus there is in addition to the amnion a fold of the blastoderm surrounding both amnion and embryo (Fig. 18). In the posterior half of the embryo the body-wall is directly continuous with the wall of the intestine as in 60. As this embryo will come up for description elsewhere, I shall not dwell further on this topic.

The fact that stands out distinctly is that the tail-fold of the amnion has not regenerated and that the head-fold has not compensated for the absence of the tail-fold by continuing its growth backwards. However, I have a number of embryos in which a complete amnion has been found without any tail-fold. These embryos, are, however, defective at the hind end, so that

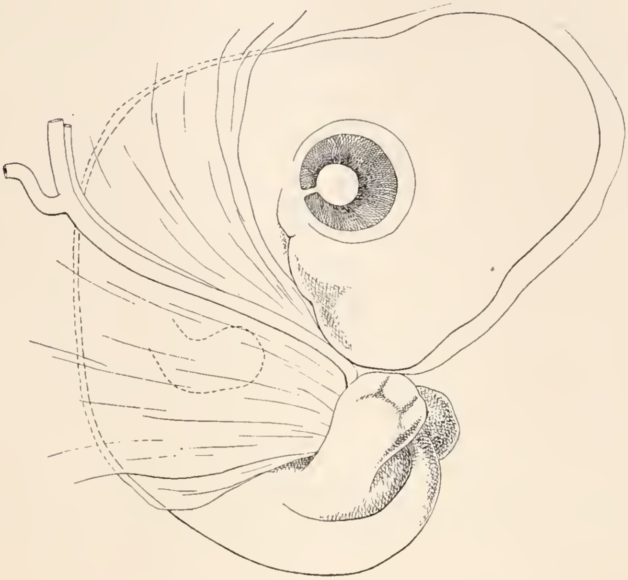

FIG. 18. Experiment 18. The embryo 48 hours after the operation. The tail-fold of the amnion has not regenerated. The amnion ends with a free edge in front of the hind-limbs. A fold of blastoderm is wrapped around the embryo and amnion. Under surface of blastoderm.

one has not to attribute any work of supererogation to the anterior lateral folds to explain the complete closure. This also will be discussed elsewhere.

GENERAL DISCUSSION.

The formation of the amnion of the chick seems to be a process with extraordinarily slight power of regulation.[1] A slight injury to part of its early rudiment sets the whole process astray. It is thus an extremely good example of correlative differentiation. The correlations in the development of the amnion are of three kinds:

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[1] Barfurth ('02) notes incidentally in one of his experiments "die Amnion war regenerirt." As I understand him, he means by this simply that an aperture made in the amnion in the course of an experiment on the eye closed up. I can confirm this from my own observations. I have found that even considerable tears made in the amnion *after its formation* may close completely.

1. *Mechanical.* — Under this head I class the elevation of the lateral amniotic folds, which takes place only after the establishment of the head-fold, and which is omitted, if for any reason the head-fold fails to appear or is destroyed.

2. *Trophic Stimulation.* — Under this head I class the influence of the traction exerted by the union of the right and left amniotic folds on the amniogenous somatopleure, and the influence of the turning of the embryo on the amniogenous somatopleure of the left side. The influence of the traction in either case is to increase the extent of the amniogenous somatopleure, in part (presumably) by stimulating its growth, in part undoubtedly by mere stretching. If, owing to failure of formation of the head-fold, such traction is not exerted on the somatopleure it does not expand nearly to the normal extent.

3. *Differential Stimulation.* — Under this head I class (doubtfully) the propagation of the ectamniotic thickening along the somatopleure; though this may be a process of self-differentiation.

Self-differentiation of the Formation of the Amnion. — The formation of the original rudiments of the ectamnion may be a process of self-differentiation, though the definite relation of the anterior and posterior rudiments to the head and tail respectively suggests correlation with their formation. Moreover, a slight histological differentiation appears between the amniogenous and choriogenous somatopleure, before, and even in the absence of, the formation of folds, which is apparently not correlated with any other of the processes observed.

Beyond this mere classification I do not desire to go at present, but will reserve a general discussion of principles until the completion of other parts of the present series.

In conclusion I simply summarize the results:

I. *Morphological.*

1. The amnion is primarily an organ of the ectoderm in the chick. The ectamnion first forms in front of the head and differentiates progressively backwards towards the posterior end,

where it is met by the posterior ectamnion differentiating forwards. Thus the amniotic zone of the somatopleure is marked off from the chorionic zone.

2. The head-fold is formed from the ectamnion with the coöperation of the amnio-cardiac vesicles and of the proamnion which is depressed between the former. The immediate prolongation of the head-fold is produced by the progressive fusion of the ectamniotic rudiments backwards, and it includes only an extremely small part of the proamnion.

3. The tail-fold is likewise formed primarily by the ectamnion with participation of the amnio-allantoic enlargements of the body-cavity.

4. There are certain constant secondary folds in the upper (right) limb of the amnion produced by the turning of the embryo. These persist longest in the region of the heart and immediately behind it.

II. *Experimental.*

1. Destruction of the anterior ectamniotic rudiment of one side prior to the formation of the head-fold of the amnion results (*a*) in permanent absence of the amnion back to the hind-limbs (exp. 57); (*b*) in inhibition of the growth, and almost complete suppression of the folds of the amniogenous somatopleure of the uninjured side; from which we may conclude —

2. That the growth of the amniogenous somatopleure is normally induced by the traction exerted on it by the progressive fusion of the folds, and that the uprising of the folds is due to the lifting power of the same process of fusion.

3. The tail-fold and posterior lateral folds cannot replace the anterior lateral and head-folds, nor can the latter replace the former.

4. Not only the initiation, but also the progress of the formation of the anterior lateral folds is dependent upon the perfection of the head-fold (exp. 36).

5. The absence of the amnion has, at least for a time, only a limited effect on the development of the allantois.

6. Inasmuch as the embryo may develop perfectly normally to the stage of five or six days without the amnion, it is obvious that the functional significance of the latter must be slight during

this period. It yet remains to be determined how far the embryo may develop without the amnion (see quotation from Dareste below).

7. There is a certain relation of interdependence between the formation of the amnion and the body-wall. In the absence of normal formation of the lateral folds of the amnion the closure of the somatopleure to form the body-wall proceeds more slowly than usual.

Dareste ('79) has observed total absence of the amnion in embryos of the chick. The condition was not, however, produced experimentally. His observations and conclusions are given in the following quotations :

" J'ai signalé, depuis longtemps, l'arrêt de développement de l'amnios et les anomalies nombreuses que cet arrêt partiel détermine chez l'embryon. C'est la cause la plus fréquente des monstrouosités simples. Il y a des cas, beaucoup moins nombreux, il est vrai, dans lesquels l'amnios fait complétement défaut. L'embryon est alors en continuité directe, par son enveloppe cutanée, avec le feuillet séreux du blastoderme, qui ne s'est pas plissé pour former la poche amniotique. J'ai vu, dans plusieurs de ces cas, l'embryon se constiteur d'une manière parfaitement normale. La paroi thoraco-abdominale s'était complétement formée, et la continuité de l'embryon avec le feuillet séreux constituait une sorte de cordon ombilical. L'allantoïde sortant de l'abdomen par se cordon s'était engagé entre le feuillet séreux et le feuillet vasculaire."

" Les embryons, ainsi privés d'amnios, peuvent vivre pendant un temps assez long. J'ai constaté l'absence compléte de l'amnios sur en embryon de treize jours, qui était plein de vie et parfaitement normal. Rien ne pouvait faire penser qu'il mourrait prochainement. Il est très-probable cependant qu'il n'aurait pas atteint l'époque de l'éclosion. L'absence de l'amnios aurait mis obstacle au développement complet de l'allantoïde : ce qui aurait produit l'asphyxie de l'embryon, comme je l'ai montré depuis longtemps. Le plus ordinairement l'absence de l'amnios amène la mort précoce de l'embryon. Souvent aussi elle détermine, dans son organisation, des modifications tératogéniques profondes."

"Toutes des observations nous font connaître le rôle physiologique de l'amnios dans la vie embryonnaire. Il est bien évident que l'amnios protége l'embryon contre toutes les actions mécaniques qui tendraient á le comprimer."

Hull Zoölogical Laboratory, University of Chicago,
April, 1903.

POSTSCRIPT.

After the foregoing paper was fully printed, my attention was called to an article by Weldon in which anamniote embryos of the fowl were described, and which I had overlooked owing to the fact that the observations were included in an article entitled "Prof. de Vries on the Origin of Species" (*Biometrika*, Vol. I., Part III., April, 1902). Partial or complete suppression of the amnion resulted from experiments to replace the water lost by evaporation in the incubator without preventing the process of evaporation itself. "A hole was made in the broad end of the egg-shell and the subjacent membranes, into which one end of a siphon, filled with water, was fitted. The other end of the siphon was placed in a reservoir of water, and the whole apparatus placed in an incubator. In from 20 to 30 per cent. of the embryos treated in this way the amnion was largely or entirely absent after incubation for three or four days."

Weldon does not discuss the mechanics of formation of the amnion, but treats the result simply as an example of a definite relation between the environment and an extremely stable character. Apparently the immediately effective factor in the experiments was the increased pressure within the shell, which, presumably, forced the embryonic area into immediate contact with the shell membrane, and thus prevented the uprising of the amniotic folds.

F. R. L.

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CROSSOBOTHRIUM LACINIATUM AND DEVELOPMENTAL STIMULI IN THE CESTODA.

W. C. CURTIS.

In the spiral valve of the "sand shark" (*Carcharias littoralis*) taken from the Woods Holl region there is found in a large majority of the specimens examined the Cestode, *Crossobothrium laciniatum*. This genus and species was first described by Linton ("Rept. U. S. F. Com." for 1886), and in subsequent papers appearing in the same publication or in the "U. S. F. C. Bulletin," he has added further important notes, the whole making an accurate and satisfactory systematic description.

A striking feature of the species is the remarkable clearness with which the important features of Cestode structure can be demonstrated. The water vascular system, main trunks and flame-cells can be seen in the fresh specimen with the greatest ease. Almost every detail of the complicated reproductive organs is seen in well-stained whole mounts of the motile proglottids and much of this in specimens freshly prepared. The mode of using the suckers on the head, the activities of the motile proglottids and their mode of egg-laying and the development of these eggs in sea-water as far as the six-hooked embryo are all easily demonstrated. Moreover, there occurs in the cystic duct of the squeteague (*Cynoscion regalis*), a not uncommon food of the "sand shark," a tetrabothrian larva which, if not the larva of *Crassobothrium laciniatum*, probably belongs to some very closely related form. This larva, which was first described and figured by Linton ("Rept. U. S. F. Com.," 1886), is again an extremely favorable object for study.

If it is possible to obtain conclusive evidence that this tetrabothrian larva of the squeteague is indeed the larva of *C. laciniatum*, we shall have but one gap in the life history of this species, viz., the transfer to the squeteague of the six-hooked embryo which develops in the open ocean.

Such favorable material it seemed to me might present, upon careful examination, facts which would be suggestive along the line of some of the general problems involved in Cestode parasitism and development in addition to the possible opportunity for fixing the life history of this particular form. With this in mind I have been collecting all the data bearing upon the life history and during the summer of 1902 I made the first of a series of experiments in infection which I hope to continue and which may lead to more precise knowledge concerning the identity of the larva found in the squeteague.

I wish in this paper to describe the important features in the structure of the motile proglottids, its egg-laying and other activities, to give some observations on the larva from the squeteague and to discuss the view point which my study of the development in this and other Cestodes has suggested to me.

The Motile Proglottids.

When an incision is made in the spiral valve of an infected "sand shark" the Cestode is frequently found in such abundance that, as the elongated bodies and the motile proglottids writhe about in the chyle, one often wonders how there can be enough nourishment left for the host. I can confirm Linton's record, of "sand sharks" taken at different times, that in the great majority of individuals there are literally hundreds of this parasite in the spiral-valve to the exclusion of all others. When the parasites are examined in sea-water the alternate protrusion and retraction of the bothria, as described by Linton, can be observed for hours. When a scolex is compressed on a slide the flame cells of the water vascular system can be observed for a considerable time before they succumb to the abnormal conditions.

The ripe proglottids which can be pulled from the long strobilæ or found loose in the intestine are very active and constantly changing their shape. A typical outline in a partially extended condition is represented in Fig. 1, and the fully elongated condition is represented on a smaller scale in Fig. 2. At the anterior tip I have found in preserved specimens minute projections which have the appearance of cilia (*c*), but which will probably prove upon examination in the living specimen to be minute spikes similar to the larger ones on the penis (*p*).

The four ear-like flaps at the posterior end which give the strobila its characteristic appearance are frequently curled back and outward, giving the posterior end a quite different outline. In the living specimen I have frequently seen masses of sperm ejected from the penis, but my records of this do not mention

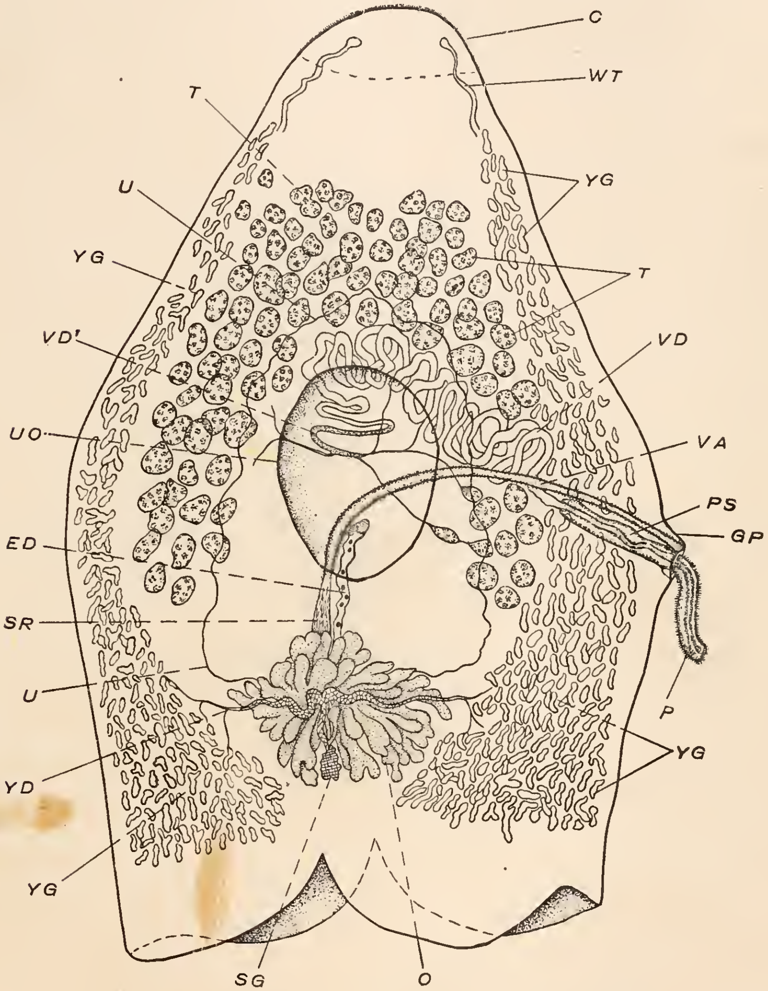

FIG. 1. Reproductive organs of a motile proglottid of *C. laciniatum*. *C*, cilia-like spikes at anterior tip; *ed*, egg duct from shell gland to uterus; *gp*, genital pore; *o*, ovary; *p*, penis; *ps*, penis sheath; *sg*, shell gland; *sr*, seminal receptacle; *t*, testes; *u*, uterus; *uo*, uterus opening through which the eggs escape; *vd*, vas deferens; *vd'*, denser inner end of same; *va*, vagina; *wt*, large water vascular tube; *yd*, yolk duct; *yg*, yolk glands.

the condition of the female organs in the proglottids thus observed. There are four main water vascular tubes. The larger pair lie on the same side of the body as the uterus opening and in the majority of cases one or both of them can be traced to a bulb-like enlargement on either side near the anterior tip of the proglottid. Posteriorly each one seems to end in the angle of the broadly wedge-shaped concavity formed by the projecting flaps. They here seem to end blindly against the cuticle which is perhaps perforated. There is no cross connection between these two vessels nor any common posterior opening such as is frequently stated to occur in *Tænias.* The second pair of main trunks are vessels of much smaller diameter and lie on the other side of the flat body immediately under the larger pair. Anteriorly they can sometimes be traced a little farther forward than the bulbs of the larger vessels, but do not seem to end in an enlargement. It is almost impossible to follow these smaller trunks for any distance posterior to the penis as the yolk glands are here closely packed together and obscure everything else.

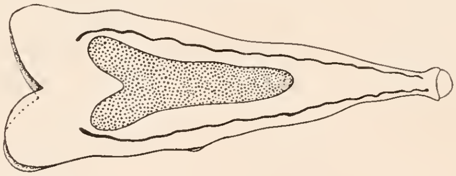

FIG. 2. Ripe motile proglottid fully extended, showing full uterus and the larger pair of water vascular trunks.

When the proglottids are examined alive much of their structure is obscured through the presence in the parenchyma of the highly refractive and closely packed granules of calcium carbonate. A very easy way of ridding the proglottid of this and preparing it for immediate examination is to use ten per cent. nitric acid and the pressure of a cover-glass. This dissolves the calcium carbonate and leaves the specimen quite transparent. This is a valuable method for the rapid examination of the principal organs, but for the finer details one of course needs more careful fixation and a good stain. I have found corrosive sublimate with about five per cent. acetic acid followed by Czokor's alum cochineal an excellent combination for the demonstration of the features given below.

The cirrus (*p*, Fig. 1) is eversible, working on the same principal as a Nemertean proboscis, a type common in Cestodes. From its base the much-coiled vas deferens (*vd*) leads away and is found throughout these coils crowded with sperm. At its inner end it has a denser wall and is of less diameter for a short distance (*vd*) and then divides into the vasa efferentia which can be seen radiating to the area in which the testes are located and in favorable cases followed down to the testicular follicles themselves (Fig. 1).

The vagina (*va*) which opens on the genital papilla just above the penis will be seen in the figure to pass inward and curve around backward, passing behind the mass of finger-like follicles which constitutes the ovary (*o*). It is here enlarged into the seminal receptacle which will be found full of sperms. Lying among the posterior lobes of the ovary is the shell gland (*sg*) to which the yolk is delivered from a common yolk duct formed by the union of a single yolk duct (*yd*) coming from either side. Extending anteriorly from the shell gland and beneath the ovary

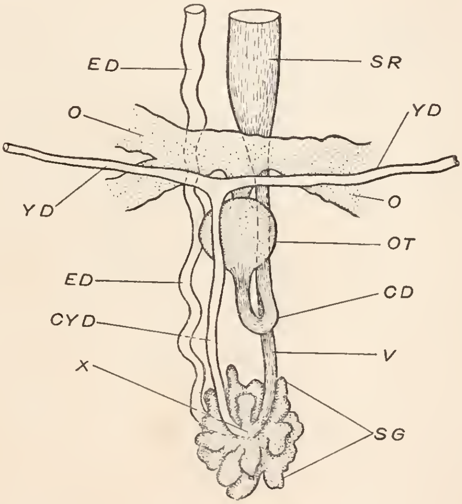

FIG. 3. Ducts of female complex with ovary lobes left out and ducts reflected slightly to show connections. *cd*, duct from oötype to inner end of vagina; *cyd*, common yolk duct; *ed*, egg duct; *o*, ovary; *ot*, oötype; *sg*, shell gland; *sr*, seminal receptacle of vagina; *v*, inner end of vagina; *x*, meeting place of ova and yolk; *yd*, right and left yolk ducts.

in this figure is the egg duct (*ed*) which conveys the eggs to the uterus. The complex of ducts in this region is shown in a reconstruction from sections represented in Fig. 3. The lobes of the ovary which are packed closely around the ducts are here omitted. This figure may be compared with what is shown in Fig. 1, where some of the same parts appear. The lobes of the ovary all converge upon a right and left portion (Fig. 3, *o*) and these main parts, on uniting, open posteriorly into a spherical cavity (*ot*) with thick walls, which is probably where the ova and sperm meet. A duct (*cd*) passes from this cavity to the inner end of the seminal receptacle (*sr*) and thence straight back to the shell gland (*sg*). Into the shell gland the common yolk duct (*cyd*) opens and from this common meeting place of the yolk and fertilized ova, after the acquisition of a shell, the fully formed egg passes up the egg duct (*ed*) into the uterus.

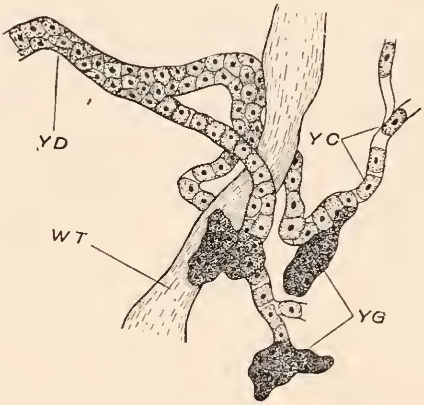

FIG. 4. Branching of yolk duct to yolk glands. *yd*, yolk duct; *yc*, yolk cells in duct; *yg*, yolk glands; *wt*, large water vascular tube.

The right and left yolk ducts branch as they reach the areas of the yolk glands on either side and some of the branches may be seen going to individual yolk follicles (Fig. 4). These main branches are often found closely wrapped around the large water vascular tubes (*wt*) of either side. Yolk cells (*yc*) may often be found on their way down these ducts and accumulated in large numbers at their median ends. They are also seen in the short ducts which run from one yolk gland to another in all parts of the mass. The yolk-producing organ consists of follicles densely packed with yolk cells and distributed in the proglottid as the figure indicates (*yg*, Fig. 1).

Fig. 1 represents a specimen killed under pressure and in which the uterus had been ruptured and the eggs squeezed from the oval hole represented by the dark outline in the center of the proglottid. Very much the same sort of a hole is left when the proglottid ruptures itself at this point in the normal egg-laying. The extent of the uterus cavity is indicated by the outline (*u*) in the figure. The condition of the intact uterus and the place of its rupture will be explained in describing the egg-laying of the ripe proglottids.

Activities of the Motile Proglottids.

The proglottids of *Crossobothrium laciniatum* are an extreme case of what is usually termed the "motile" condition. So definite are their movements and activities that one is constantly thinking of them as though they were individual animals of a species entirely distinct from the parent scolex. When observed in the chyle they are seen writhing about, contracting and elongating rhythmically and bending their bodies into an arch along the axis of breadth, now one way, now another. If we measure the maturity of a proglottid by its size and the number of eggs accumulated in the uterus the conclusion is reached that the proglottids as taken from the spiral-valve are of diverse ages, for one finds a considerable variation in the number of eggs accumulated in the uterus and a correlated variation in the size of the proglottids.

When placed in clean sea-water the smaller proglottids do not lay their eggs, while the large ripe ones will almost immediately do so. These facts seem to indicate that the proglottids may be shed off from the strobila some time before they are ripe and remain in the shark's intestine until they are fully loaded with eggs and ready for the laying. The enormous number of proglottids usually found in a single spiral-valve is another fact in favor of this conclusion. On the other hand fully mature proglottids are frequently found on the end of a strobila (Fig. 5), showing that they may mature while still attached to the parent stock.

When carefully examined the ripe proglottids at the posterior end of a strobila (Fig. 5) or the mature motile proglottids found free in the chyle show a breast-like protuberance upon that face on which the uterus opens. The resemblance of this to a breast is heightened by the existence of a nipple-like prominence at the summit, as is shown in the side view given in Fig. 5. The general protuberance is caused by the distension of the uterus, though it sometimes seems to be enhanced by a concavity on the opposite face of the segment as the dotted line of the figure indicates. Motile proglottids when in this ripe condition show, if examined in the chyle, the ordinary writhings and indefinite locomotor movements noted above. If, however, a number of these ripe and full proglottids are transferred from the chyle into clean sea-water the egg-laying will presently be observed. In making observations on this process I was accustomed to select carefully ten or a dozen proglottids which seemed fully ripe and transfer them all together into a dish of clean sea-water. When this was done it was found that about eight out of ten thus selected laid their eggs in three or four minutes. Any of those remaining might lay after a little longer period or not at all. A similar reaction of whole chains of proglottids is recorded by Schauinsland (Jena, Zeitsch. 1886), for *Bothriocephalus latus*, *Trianophorus nodulosus* and *Ligula simplissima*.

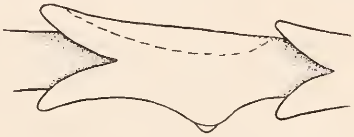

Fig. 5. Side view of a ripe proglottid.

When proglottids of *Crossobothrium* are taken at random and thus placed in sea-water only a small proportion, no more than one fourth or one fifth, will ever lay their eggs. When the small proglottids which have only a few eggs in the uterus are thus taken no egg-laying follows in any case.

That the proglottids as found in the spiral-valve at any one time are not all of same maturity is thus clearly shown and I think we are justified in the conclusion that these immature proglottids tend to remain in the spiral-valve until they become fully ripe and then to pass out with the fæces, even though their early detachment from its strobila may have been premature and caused by the outward passage of the excreta or the contraction of the intestine.

The manner in which the egg-laying proceeds in any single proglottid thus placed in sea-water is a very interesting thing to watch. Extreme writhing movements of a quite definite sort begin at once. The proglottid bends along its axis of breadth until it is almost a closed ring, the pointed anterior end sometimes passing into the angle made by the posterior flaps (Fig. 2) and thus reminding one of an acrobat who could bend backward until his head should be thrust between his legs from behind, then the proglottid straightens and the bend is reversed, it straightens again and bends into the first position and so on; these motions continue until the nipple-like prominence of the protruding body bursts and the liberated eggs gush forth. When the break occurs the extreme violence of the writhing ceases and the proglottid bends backward rather more than forward (Figs. 6 and 7) until all the eggs have been expelled, when a gaping hole is presented (Figs. 6 and 7) where once was the distended uterus. Even when there is hardly an egg left within it, the straining movements of the proglottid continue as though it were making sure that not a single egg remained. Figs. 6 and 7 represent proglottids in which the egg-laying had been almost

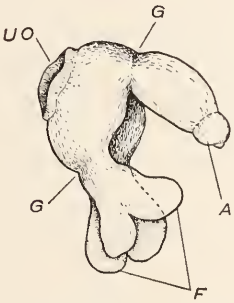

FIG. 6. Proglottid in the act of egg-laying seen from the side. Lettering same as for Fig. 7.

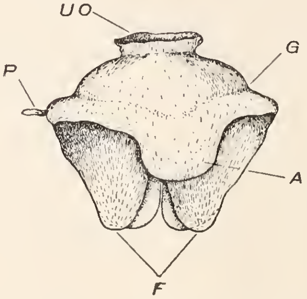

FIG. 7. Proglottid at close of egg-laying seen from anterior end. *a*, anterior end; *p*, penis; *g*, groove marking outline of uterus; *uo*, ruptured area through which the eggs escape.

accomplished and show the characteristic attitude of the proglottid from a front and side view.

Proglottids which have thus stripped themselves of their eggs may continue to live in sea-water for a day or two, but I have not experimented with them to ascertain how long their existence may be prolonged.

The rupture of the uterus may be very readily produced in a proglottid having any considerable accumulation of eggs, if a little pressure is applied with a cover-glass or otherwise. But the process above outlined is something brought about spontaneously by the proglottid itself after it is transferred to sea-water. Proglottids which have been artificially compressed in killing, for whole preparations almost always have the uterus ruptured and the eggs discharged. They then present the appearance shown in Fig. 1, of a large oval hole opening into the uterus cavity, while the boundaries of the latter can be traced as a very delicate outline still conforming to the general outline of the full uterus. A proglottid which is in the last stages of egg-laying after the spontaneous rupture of its uterus shows the same sort of opening, but perhaps more widely distended. Such specimens which were killed without compression are shown in Figs. 6 and 7.

My observations are then that the proglottids when large and having the uterus full of eggs (Figs. 2 and 5) will, by a quite definite series of muscular contractions and writhings, rupture the nipple-like prominence at the summit of the protruding uterus (Fig. 5) and allow the eggs to gush forth, the proglottid continuing its writhing movements in a less pronounced degree even after all the eggs have been shed (Figs. 6 and 7). The fact that this egg-laying occurs immediately after the ripe proglottid is transferred from the chyle to clean sea-water will, I think, convince any one that the same process occurs when a ripe proglottid of *Crossobothrium* passes in the normal course of its existence out of the shark's cloaca into the water of the ocean. We may conclude I think that such a proglottid, upon coming into contact with the sea-water outside, goes through muscular contractions similar to those observed in the laboratory and lays its eggs free in the open ocean, and that these pelagic eggs are thus widely scattered. The short period between the first contact with the outer water and the egg-laying indicates that the infection of the intermediate host is, by means of countless embryos, developed in the open ocean and not by the eating of the intact proglottids with their contained eggs.

EGG DEVELOPMENT.

On collecting the eggs laid by proglottids in the laboratory and placing them in dishes in which the sea-water can be kept reasonably pure, development ensues as far as the six-hooked embryo stage which I have represented in Fig. 8, drawn from the living specimen. I did not succeed in obtaining embryos beyond this stage, and therefore cannot say whether the embryo enters the next host in this condition or as a ciliated larva (Schauinsland, "Bothriocephalidæ," '86) which subsequently hatches from the embryo figured.

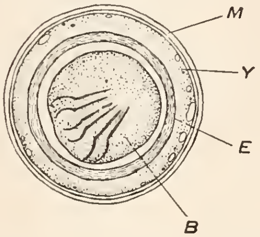

FIG. 8. Six-hooked embryo of *Crossobothrium laciniatum*. *m*, egg membrane; *y*, remains of outer envelop; *e*, ectoderm of Schaunisland; *b*, six-hooked embryo.

In the common Tænias and those Cestodes which have similar hosts and conditions of life-history, the fertilized eggs on passing into the uterus develop *there* into six-hooked embryos and remain in that stage until they reach the tissues of the intermediate host. In the Bothriocephalidæ, Schauinsland describes *B. rugosus* as having such an intra-uterine development as far as the six-hooked embryo, and *B. latus*, *Trianophorus nodulosus* and *Ligula simplissima* as producing eggs which develop only when they reach the water. In the last three species the eggs accumulate in the uterus in the condition of resting oösperms surrounded by their yolk and the egg-shell, but do not develop until they are laid in the water by the parent proglottid after this has left the host's intestines.

My material was prepared for the general anatomy rather than for this particular point, and the egg capsules are greatly shrunken, but as nearly as I can make out from a careful examination of the uterine eggs they are all in the condition above noted, viz., a resting fertilized ovum plus the yolk cells and egg capsule. In Cestodes having this mode of development, therefore, the eggs accumulate in the uterus, but do not develop until they are laid by the proglottid. The resting stage is comparable to the resting condition known in many forms which lay winter eggs or eggs which develop only after a considerable time. In any given proglottid such Cestode eggs are of diverse ages, depending upon how long they have been in the uterus, but are all alike inhibited from development until the proper conditions are present. Upon contact with the external water this resting stage is stimulated, or, we might say, some inhibition is removed, and development ensues, continuing as far as the six-hooked embryo. Whether there is some specific thing in the sea-water which can be fixed upon as the stimulus to development I have not ascertained, but it has seemed to me quite possible that the stimulus can be located in some specific chemical constituent of the sea-water.

From what we know of other Cestodes it is unlikely that the six-hooked embryo of *Crossobothrium* can develop further in the water, and that if this embryo does not find the appropriate stimulus for further development by meeting with its next host it must perish. What this next host is one would perhaps discover as much by accident as by the most persistent work. Could I clearly demonstrate that the larva found in the squeteague is the young of *Crossobothrium laciniatum* — and there is a good deal ot general evidence that this is the case — I think that the nature of the squeteague's food (young herring, adult herring, menhaden, etc. — Peck, "Sources of Marine Food," U. S. F. C. Bull., '95) would lead us to suspect that the six-hooked embryo, instead of passing directly to the squeteague, might have an intermediate host like the menhaden, herring, or some fish which feeds upon the microscopic elements in the sea-water.

Developmental Stimuli in the Cestoda.

To one examining closely the present trend of opinion regarding the process which our nomenclature still designates as fertilization it is, I think, quite apparent that the evidence and conclusions so logically and convincingly set forth by R. Hertwig[1] are gaining a wide acceptance both among special workers and among those who are viewing the data with a somewhat broader perspective. The details of Hertwig's paper had been already accepted by some of the investigators whom he cites, and so far as they concern the Protozoa they are mentioned by Calkins in his recent book as well-established facts, but this cannot detract from the able manner in which Hertwig has summarized these facts and indicated the important conclusions to be drawn from them. The work which to most persons offers convincing evidence of the twofold nature of the process we have been calling normal fertilization is the work in "artificial parthenogenesis" initiated by Loeb and Morgan. One could hardly ask for a more convincing proof that the union of the germ plasms and the "developmental stimulus," as Hertwig calls it, are distinct and separable phenomena although they have become almost indissolubly connected with one another throughout the Metazoa.

It having been shown in these experiments so far as they have now gone that a stimulus to development may be operative entirely independent of any union of the germ-plasms, as for example in the development of eggs of echinoderms or worms upon treatment with salt solutions and other stimuli, we should I think seek no less for the converse proposition, viz., *the union of the germ-plasm and the absence of developmental stimulus* (as evidenced by the absence of development) *while the oösperm continues its life*. We should then seek for the proper stimulus to this resting condition and by means of this stimulus initiate at will the developmental changes. To illustrate by a hypothetical case, suppose we could have a form where it were possible to bring about at will the union of the ovum and spermatozoön and their nuclei but by subtracting the "developmental stimulus" to have the resulting oösperm go into a resting state which would result eventually in its death unless the "stimulus to development" intervened to start a new cycle. If this developmental stimulus, or some substitute for it, and its action were accurately known we might apply it at will at any time after the union of the germ-plasms was completed, and so long as the oösperm remained alive it would respond by beginning the new cycle of development.

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[1] "Mit welchen Recht unterscheidet man geschlechtliche und ungeschlechtliche Fortpflanzung?" *Sitz. Ber. Gesel. f. Morph. und Physiologie in München*, Nov., 1899. Translated in *Science*, N. S., Vol. XII., no. 312, Dec. 21, 1900.

In the thorough understanding of the two apparently distinct processes involved in fertilization as hitherto understood we should I think be greatly aided by the investigation of the problem just outlined which is the *converse* of that brought out by the work on "artificial parthenogenesis."

We have, it seems to me, cases of normal development which are parallel to this converse proposition in just the same way that normal parthenogenesis is a parallel to the artificial development induced by salt solutions and other stimuli. These cases are seen in the development of those forms in which the fertilized egg has a long resting stage. Where freezing or desiccation is necessary for such subsequent development, this condition may be regarded as one of the developmental stimuli, although if one tries to picture how a state of affairs necessitating this particular condition could have arisen in the past he must, I think, feel certain that though extreme cold or dryness may now be a necessary factor it must originally have been unnecessary if not a thing fatal to the further existence of the organism.

Any one familiar with biological literature can readily recall numerous cases of eggs with longer or shorter resting periods following upon the union of the germ plasms. The particular case I call attention to is that presented by those Cestoda which in their development have *resting fertilized eggs* gradually accumulating in the uterus as in the case in *Bothriocephalus latus*, *Trianophorus nodulosus*, *Ligula simplissima*, etc., and in *Crossobothrium laciniatum*. On referring to the description which has been given of the production of the eggs and their extra-uterine development it will be seen that this illustrates particularly well what I have termed the converse of artificial parthenogenesis and that the hypothetical case which I set forth on page 137 might be substituted almost word for word for a description of what occurs in one of these Cestodes.

In addition to the phenomenon of a resting stage in their early development the Cestodes just mentioned, and indeed all other Cestoda, present in their subsequent life history a feature which I have found interesting when considered in connection with the primary "developmental stimulus" which starts the oösperm upon its course. Such a consideration of the subsequent facts of Cestode life history may perhaps widen our conceptions regarding the nature of one of the two phenomena which exist side by side in normal fertilization. How this is will be easily apparent if we recall the life history of *Crossobothrium* or any Cestode having a similar extra-uterine development.

The female reproductive organs of each proglottid produce ova which on being fertilized become surrounded by their yolk supply and encased in a tough shell. Without undergoing any developmental changes they accumulate in the uterus where they remain in this condition until the time of egg-laying. They are thus of very diverse ages if we date the age of each from the time of the entrance of the spermatozoön, but all are in the same *resting unicellular state.* We have here the union of the germ plasms, but the stimulus to development delayed for a period which is long or short, depending upon the age of the individual oösperm. The stimulus to development is normally found in the contact with the outside sea-water when the eggs are shed, for the cleavage begins only when they are thus set free. Development proceeds so far as the six-hooked embryo stage when death ensues unless the proper host is found. In the case of *Crossobothrium* there is perhaps a primary intermediate host between the six-hooked embryo and the squeteague in which case the six-hooked embryo which infects this intermediate host receives a stimulus to development which sends it so far as the resting stage which is attained in that particular host, and here it stops and eventually comes to naught unless it is carried into the next host, the squeteague, where it finds a new stimulus to further developmental changes and attains in the cystic duct of this fish its development to the full structure of a tetrabothrian larva.

Here again death ensues unless the next stimulus in the series is forthcoming, viz., the contact with the digestive juices of the "sand shark's" stomach. When this stimulus is furnished the tetrabothrian surviving the wreck of its teleost host develops into the final adult condition.

The foregoing is stated in sufficiently general terms to be applicable, *mutatis mutandis*, to any Cestode having an extra-uterine development and whether or not the life history outlined for *Crossobothrium* is the correct one does not affect the general conception of Cestode life history which I am attempting to portray.

In stating the above I have spoken of the reaction of the embryo of a given stage to its particular stimulus just as I spoke of the reaction of the oösperm to the stimulus which initiates the whole cycle. If we ask ourselves what is the essential nature of the reaction of the oösperm to the delayed developmental stimulus we must designate it as primarily a reaction manifest in so many cell divisions and subsequent differentiations. In what does the result produced by any one of the stimuli, which, if the embryo runs its whole course, become applied to each of the successive stages, differ from the result produced by the stimulus which acts upon the oösperm? Cannot the result in each instance be formulated in the same way, viz., that the stimulus causes cell divisions and subsequent differentiation? And should we not speak of all of them as "developmental stimuli"?

The exact nature of the stimulus at each stage is a thing which in spite of many technical difficulties would be open to investigation and something which we may hope eventually to understand, but however diverse the stimuli might be we should still have, as above stated, changes of the same nature resulting from each successive stimulus. The stimulus which is in the first instance something in connection with the sea-water is probably in the other cases a change in nourishment incident to the change of hosts, but in every case the *result* of the stimulus may be stated in the same general terms, viz., cell division and subsequent differentiation which ends in a condition of stable equilibrium in which the animal finally perishes unless the next stimulus is forthcoming.

Viewing then the life history of such a Cestode from this point of view we have first the union of the germ plasms followed by a resting stage of varying duration. A stimulus furnished by the contact with the sea-water when the eggs are laid brings about the changes resulting in the six-hooked embryo. This embryo when it receives a certain stimulus (condition of nourishment or otherwise) from the intermediate host goes as far along the course of development as the mid-larval stage and stops again. On reaching the stomach of the final host the last stimulus of the series is furnished and the adult condition attained.

In those Cestoda which have an intra-uterine development, *i. e.*, forms in which a six-hooked embryo develops in the uterus, we find the primary developmental stimulus intimately associated with the fusion of the germ plasms as in the fertilization of most Metazoa, though in *Bothriocephalus rugosus* (Schauinsland, '86) the intra-uterine development may not begin for several weeks after the eggs have begun to pass into the uterus cavity. In such cases the comparison between the primary "developmental stimulus" and the developmental stimuli which follow is of course not so patent, although it is none the less legitimate. Whether what I have called a "*developmental stimulus*" in these several cases shall be found eventually to be some new condition which the egg or embryo meets or be found to be the removal of some existing condition which has been inhibiting the development is of no consequence here since the removal of an inhibition may be spoken of as a stimulus, and since the important thing is not the nature of the stimulus but the similar reaction of the animal in each case.

In conclusion I may say that no facts not already familiar to students of Cestode life history have been set forth in the section of this paper just concluded, nor can it be claimed that the apparently two-fold nature of fertilization has not been in recent years more than once promulgated. The comparison between the reaction of the oösperm to the primary developmental stimulus and the reaction of the larval stages each to its special stimulus has interested me and it has seemed to me worth while to attempt the formulation of Cestode life history from this point of view. I also believe that the two-fold nature of fertilization has not yet reached such a point of general acceptance or rejection, but that it will bear further illustration in any case in which there are facts particularly germane to the question and with these two points in view I have attempted the foregoing formulation.

University of Missouri,
Columbia, Mo., May 1, 1903.

Vol. V. *August, 1903.* *No. 3*

BIOLOGICAL BULLETIN.

ON THE CONDITIONS GOVERNING THE PRODUCTION OF ARTIFICIAL PARTHENOGENESIS IN ARBACIA.

S. J. HUNTER.

In a previous paper[1] it was shown that sea-water concentrated by evaporation to a definite volume would produce parthenogenesis in eggs of the sea-urchin, *Arbacia*, subjected to its influence for a given length of time. During the continuation of these experiments for the purpose, primarily, of observing the morphological phenomena there has become evident a series of conditions necessary to a high ratio of parthenogenetic development. These conditions are briefly — purity of solutions, stage of development of ovarian eggs when placed in the concentrated sea-water, length of time the eggs are to remain in this solution, temperature of solutions. This article is based upon observations on eighty-three experiments, fourteen of them performed between July 25 and August 14, 1901, and sixty-nine between July 4 and August 15, 1902. The work of both seasons was pursued at the Marine Biological Laboratory, Woods Holl. In every instance the eggs for each experiment were taken from one female only. In this brief preliminary account detailed references to individual experiments are in some cases given, not to illustrate the condition peculiar to that experiment alone, but rather to set forth prevailing conditions.

Solutions.— These are rendered ineffective, (1) by the presence of foreign substances; (2) spermatozoa; (3) excessive number of eggs. The eggs of *Arbacia* are extremely sensitive to foreign substances liable to be introduced by the use of glassware not thoroughly cleansed or glassware previously used as receptacles for chemicals. For this reason it was found advisable to use only new glassware.

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[1] Hunter, *American Journal of Physiology*, VI., 1901, p. 177.

Much time was consumed in the work of sterilization to prevent contamination from spermatozoa. This, however, is essential. In the eighty-three experiments referred to sterilization was performed in accordance with the plan mentioned in the paper cited. If, after this treatment, there were eggs that developed through normal fertilization, such escaped notice. In five other experiments made to determine the necessity of this sterilization, the sea-water was not sterilized nor was the sea-urchin carefully washed in hydrant water. In these five experiments a few normally developing forms were noted.[1]

The relative proportion of eggs to concentrated sea-water is an important factor in determining the percentage of development. In the paper referred to mention was made of the necessity of placing comparatively few eggs in the solution. Tabulated results of some experiments on this point make the relative value of this condition more apparent. In two bowls containing equal amounts of condensed sea-water there were placed in the first bowl a greater number of eggs and in the second bowl very few. Out of the first bowl 14 per cent. reached the swimming gastrula stage; out of the second bowl 87.5 per cent. reached the same stage. Notes taken on early stages of the culture showed that in the bowl containing the less number, segmentation began in a greater number of eggs.

In other experiments an endeavor was made to measure approximately the number of eggs placed in a given amount of sea-water. One of these experiments is placed in tabulated form.

No. 1, 1 pipette full of eggs in 50 c.c. concentrated sea-water.
No. 2, 5 pipettes full of eggs in 50 c.c. concentrated sea-water.
No. 3, 10 pipettes full of eggs in 100 c.c. concentrated sea-water.

After two hours, transferred to sterilized sea-water, frequently changed at first — examined twenty-seven hours later with results as follows:

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[1] The test of purity of culture was based on the facts, (1) that cleavage in parthenogenetically developing eggs of *Arbacia* at no time prior to blastula stage, resemble normal processes; (2) the shortest time in which any culture under constant observation reached the active swimming stage was nine hours and seven minutes, under like conditions normally fertilized embryos become active in about six hours; (3) the absence of the perivitelline membrane.

No. 1, 7 gastrulæ out of 16, 43 per cent.
No. 2, 9 gastrulæ out of 90, 10 per cent.
No. 3, 10 gastrulæ out of 109, 10 per cent.

Examined again twenty-six hours later with the following results :

No. 1, 8 plutei and 12 gastrulæ out of 27, 74 per cent.
No. 2, 1 pluteus out of 42, .02 per cent.
No. 3, no living forms out of 39.

This difference in the ratio of development is probably due to the noxious effects of the undeveloping eggs in the solution. This being the case a frequent change of the concentrated solution might raise the percentage, for while the sterilized sea-water was changed repeatedly the concentrated water was not changed at all during the period. The difference in results was evident at this stage, that is, the two lots of eggs when removed from the condensed solution showed a difference in behavior. As just noted, the eggs from the cultures containing the smaller number showed a larger percentage of segmentation. Cultures No. 2 and No. 3, having same ratios, gave similar percentages.

State of Development. — Wilson [1] has observed that the eggs of *Toxopneustes* which would not fertilize with spermatozoa gave some of the best results obtained with the magnesium solution. Delage [2] notes in *Strongylocentrotus* that frequently eggs which will not develop by artificial parthenogenesis are readily fertilized by spermatozoa. A number of observers have noted the wide variation in the behavior of the eggs of different females. Some eggs do not develop at all, others give large percentages of active larvæ. A case in point : An experiment with a large female, the eggs of which by their number, color and the freedom with which they came from the ovary — the ovaries in such cases when placed in sea-water lose form and become a mass of eggs — seemed to signify that the eggs were fully matured (oötids). As further proof, the greater part of these eggs were fertilized with spermatozoa, this resulted in the normal development of all the eggs observed. The remainder of the eggs were subjected to the influence

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[1] E. B. Wilson, "A Cytological Study of Artificial Parthenogenesis in Sea-urchin eggs," *Archiv f. Entwickelungsmech.*, XII., 4, 1901, p. 535.

[2] Delage, Y., "Études Expérimentales sur la Maturation Cytoplasmique Chez les Echinodermes," *Archiv d. Zoöl. Exper. et Generale*, 3, Ser., IX., 1901, p. 300.

of condensed sea-water for two hours and four minutes, resulting in the appearance of many cytasters (Wilson) and the segmentation of many eggs, but no active larvæ. Segmentation and development ceased after four hours in the sterilized sea-water. The ovaries of another female were teased in sea-water and a small number of pale eggs were obtained. From the condition of the ovaries, the color and the number of eggs it was evident that the eggs were not mature (oöcytes). These were placed for the same length of time in concentrated sea-water and then transferred to normal sea-water. More than 90 per cent. of these eggs reached the active larval state. Eggs of females brought directly from the bed of the ocean gave better results than those kept in the laboratory aquarium for a time. The higher temperature of the sea-water in the laboratory probably hastened maturation. It became evident throughout the later series of experiments that oöcytes gave satisfactory results and oötids gave negative results. It seems probable, therefore, that concentrated sea-water is effective in producing development in *Arbacia* only when its influence is brought to bear upon the oöcyte.

The interesting question naturally arises concerning the exact stage at which the solution is effective. If this influence causes retention of the second polar body and its assumption of the rôle of the spermatozoön the subject is at once brought into direct relation with Boveri's[1] theory of natural parthenogenesis. In Delage's[2] observations on the influence of carbon dioxide on *Asterias* he gives results to show that the moment of susceptibility of the eggs lies between the time when the nuclear membrane of the germinal vesicle begins to dissolve and the beginning of the resting period of the egg nucleus ; and that the immediate cause does not concern the polar bodies but rather the suspension of maturation for a given period. Upon resumption the polar karyokinesis is not confined to one region of the egg, but instead becomes general and includes the whole egg. Consideration of this phase is curtailed by Delage's[3] own statement

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[1] Th. Boveri, "Zellstudien," I., 1887, p. 73.

[2] Y. Delage, "Nouvelles Recherches sur la Parthenogenese Experimentale chez Asterias glacialis," *Archiv de Zoöl. Exper.*, 1902, p. 217.

[3] Y. Delage, "Etudes Experimentales sur la Maturation cytoplasmique chez les Echinodermes," *Archiv de Zoöl. Exper.*, 3 Ser., IX., 1901, p. 295.

that phenomena manifested in the starfish must not be assumed to occur in the sea-urchin, and further according to the same author[1] the eggs of *Strongylocentrotus* are mature before being subjected to the solution. From this it would seem that there is a difference in the behavior of the eggs of *Arbacia* and *Strongylocentrotus.* In *Arbacia* it did not appear that in the development of the egg there was only one opportune moment when the concentrated solution was effective, but rather that ovarian eggs placed in the concentrated solution, were influenced to maturate and that maturation brought about in this way resulted, when the eggs were removed to normal sea-water, in segmentation and subsequent development. Experiments with eggs apparently mature frequently give small percentages, one to five per cent. of larval development. This may be accounted for by the presence of a few oöcytes in the ovaries. The difficulty in the case of *Arbacia* is that owing to the opacity of the eggs it is not possible to ascertain their exact state when placed in the concentrated solution.

There is some evidence which probably bears upon the question to be found in the examination of sections. Without entering into a detailed description at this time we find in iron-hæmatoxylin sections a heavily staining body in contact with the nuclear membrane. In some cases astral rays extend out from this dark body. In others these rays are absent. Later prophases occur, such as the elongation of the nucleus with an aster at each pole, followed by the mitotic figure in its various phases. In other words, there appear to be processes closely resembling normal karyokinesis. This conspicuous dark body shows its attitude towards the nucleus in cases where the dark body has failed to divide. In such cases, the nucleus is elongated on the side of contact and the chromatin is aggregated on the same side. It seems reasonable, therefore, to say that in these parthenogenetic eggs there is a force whose behavior approximates that of the spermatozoön.

Briefly, then, in the sea-urchin egg maturation takes place in the ovary before normal oviposition.[2]

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[1] *Ibid.*, pp. 296, 301, 324.

[2] E. B. Wilson, "The Cell," 1900, p. 236.

The eggs used in these experiments were not deposited naturally, but were from ovaries removed from the female.

The ovaries thus taken were of two kinds: first, dark red in color, delicate in structure; when placed in sterilized sea-water eggs flowed freely from them without cutting or teasing; second, light red in color, firm in structure; comparatively few eggs obtained even after ovaries are cut and teased.

The eggs from ovaries of the first class gave unsatisfactory results when subjected to influence of concentrated sea-water, satisfactory results when fertilized with spermatozoa.

The eggs from ovaries of the second category have given percentages as high as 80 to 90, of parthenogenetic swimming forms, when subjected to influence of concentrated sea-water for the proper period.

Sections through ovaries, typical of this second class, reveal large numbers of oöcytes determined as such by the presence of the prominent germinal vesicle. Sections through thirty-two different follicles were examined. Only those showing germinal vesicle (oöcytes) or egg-nucleus (oötids) were counted. In these thirty-two sections of follicles there were 183 oöcytes and 85 oötids.[1] Oöcytes much smaller than normal eggs were not counted. The percentage of forms developed parthenogenetically is thus shown to bear a direct relation to the number of oöcytes in the culture.

It seems reasonable, then, to infer that the concentrated sea-water acts effectively upon the oöcyte only. The exact nature of this action it is hoped subsequent study will determine.

Duration.—The eggs of *Arbacia*, as is well known, are not sufficiently transparent to permit close observation upon the activity of the cell contents. For that reason I have been unable to note in the egg any definite appearance which would signify the proper moment for transference from condensed sea-water to sterilized sea-water. In a few cases I have found wide variations in the time that the eggs can be transferred and yet develop. The shortest time was one hour and twenty-two minutes. The limits within which the eggs from a given culture could be removed and yet develop were relatively narrow. It seems that the critical moment does not lie, as in the case of Delage's observations on *Asterias*, in the time when the eggs are placed in the solution, but rather when they are removed from the solution.

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[1] These follicles were from the ovaries of one female. Of the utilized eggs from this female fully 75 per cent. became swimming larvæ.

The stage of development of eggs when placed in solution evidently has some bearing on the time required for development. The differences in the states of ovarian eggs would seem to account for the differences in the time required for development, not only for the eggs of different individuals, but as in the experiment given below, for the eggs of the same individual.

The culture just referred to, the one in which larval development was obtained after an hour and twenty-two minutes in the concentrated solution, was one of a series of experiments to determine the proper length of time and also the time in which eggs of a given female will develop. This experiment also presented the longest period of time within which eggs of the same individual could be removed from the concentrated solution for subsequent development. The eggs were placed in the concentrated sea-water and allowed to remain one hour. Watch-glass cultures of approximately equal number of eggs, the standard being three pipette drops of eggs in each watch glass, were removed every two minutes from the concentrated solution and placed in sterilized sea-water. The length of time that eggs remained in the concentrated solution is given and opposite are the observations made, beginning seven hours and twenty minutes later.

Minutes in Concentrated Solution.	Notes Taken Seven to Ten Hours Afterward.
62.	No segmentation.
64.	A number of fragments from a few eggs that had segmented and then broken.
66.	The same.
68.	The same.
70.	Fragments more abundant but nothing in the nature of a cluster of blastomeres.
72.	The same.
74.	The same.
76.	Not so many fragments, a few eggs segmented into two and three blastomeres.

78. Very few whole eggs, nearly all in fragments of halves and less sizes.

80. Blastomeres remaining together, few fragments.

82. Three active well-formed blastulæ (examination made ten hours after removal from concentrated sea-water).

82–98. The eight cultures taken out during this time showed about the same percentage of development as 82.

100. No segmentation in this culture nor in any of the subsequent cultures.

This experiment shows a duration of sixteen minutes within which eggs were removed and larval development ensued. This was the widest range of the series. In many experiments a difference of five minutes on either side of the optimum moment determined the life of the culture. In all cases, as noted by other observers, eggs removed from the concentrated solution after a brief period begin to segment but do not continue to develop until they reach the swimming blastula stage. Eggs permitted to remain too long plasmolyze when placed in sterilized sea-water. As a result of this series of experiments the optimum period was determined at two hours. In each case three cultures were formed of the eggs, one of five minutes before the period, one at the period, and the other five minutes after the two hours.

Temperature. — The most favorable temperature obviously is the normal temperature of sea-water. Sudden changes caused by the use of water of a different temperature for replenishing cultures is detrimental. Greeley[1] has shown that blastulæ can be developed parthenogenetically in concentrated sea-water at a temperature of 2°, 11° and at the room temperature of 23°. I am convinced that uniform results cannot be obtained from cultures kept on the laboratory table. The changes in temperature which occur between day and night materially affect the behavior of the eggs. For this reason towards the close of the season the bowls containing the solutions were surrounded by running sea-water. This insures constancy of temperature as well as approximates the normal temperature.

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[1] A. W. Greeley, Biol. Bulletin, IV., No. 3, p. 132.

SUMMARY.

1. The conditions governing the production of artificial parthenogenesis in *Arbacia* by the use of sea-water concentrated by evaporation to a definite volume, are purity of solutions, stage in development of ovarian eggs, duration in concentrated solution, temperature of solutions.

2. The efficacy of solutions is subject to the presence of foreign substances, spermatozoa, relative number of eggs in a given amount of concentrated sea-water, and temperature. Foreign substances are excluded through extreme care in the preparation of solutions; spermatozoa are eliminated by raising normal sea-water to 70 degrees, by sterilizing all instruments in the flame, by washing thoroughly the body of the sea-urchin and the hands of the operator for three brief periods under stream from the hydrant. Results are most constant at normal temperature of sea-water. Development is obtained at room temperatures 22° to 24°. Variations in temperature of solutions materially affect the development of the culture.

3. The concentrated solution appears to be effective in producing development in oöcytes only. By reason of the opacity of the egg it is difficult to ascertain the exact stage or subsequent behavior in concentrated solution.

4. The average optimum period for eggs in concentrated solution lies between one hour and fifty-five minutes and two hours and five minutes.

University of Kansas,
April 4, 1903.

HETEROGENY AND VARIATION IN SOME OF THE COPEPODA OF LONG ISLAND.

ESTHER F. BYRNES.

In the spring of 1898, my attention was attracted to certain of the Copepoda that occur in large numbers in the fresh-water ponds in some of the outlying districts of Brooklyn. The material, which contained many *Cyclops*, was collected soon after the ice had disappeared from the surface of these shallow pools and even at this early season most of the *Cyclops* were large and carried eggs in all stages of development.

I isolated individuals with eggs, and subsequently observed numerous color-changes, which accompanied the rapid growth and extrusion of eggs into the egg-sacs. A single instance will suffice to show the rapidity of these changes, and the fertility of the individuals. On the 19th of April, 1898, a *Cyclops*, carrying dark blue eggs, was isolated. On the 20th dark bluish ova could be seen through the transparent body-wall, making the body appear dark, while the dark eggs in the egg-sacs had developed into embryos of a reddish tint. On the 22d the copepod carried dark eggs again, and the body was again almost colorless, with a faint streak on either side, still marking the position of the ovaries. On the 23d it remained unchanged. On the 24th the body was again dark but no eggs were attached. On the 25th the dark eggs were carried in appended sacs and the body was again colorless. On the 26th the dark eggs became detached. On the 27th the body again appeared dark. There is no record in my notes for the next two days, but when I again looked at the copepod the body was colorless. While it carried no egg-sacs, the ova must have been discharged since the last record on the 27th instant.

I attempted to identify the form, which agreed with *C. parcus* (Herrick), in most of the points that are regarded as species-characteristics but it differed from *C. parcus* in the number of its antennal segments.

The chief morphological features by which species of *Cyclops* are recognized are the following :

1. The number of joints in the antennæ.
2. The number of joints in the rami of the four swimming feet.
3. The armature of the swimming feet.
4. The number of joints in the fifth foot, which is rudimentary.
5. The shape and armature of the segments of the fifth foot.
6. The structure of the abdomen with the caudal stylets and the armature of the caudal stylets.
7. The shape of the receptaculum seminis.
8. The armature of the maxillipeds.
9. The relation between the length of the antennæ and the cephalothorax.

The characteristics of *C. parcus* are as follows :

1. Seventeen-jointed antennæ.
2. Three-jointed rami in the swimming feet.
3. Armature of the last segment of the swimming feet.

First Foot.		Second Foot.	
Outer Ramus.	*Inner Ramus.*	*Outer Ramus.*	*Inner Ramus.*
2 outer spines.	1 outer seta.	2 outer spines.	1 outer seta.
2 apical setæ.	1 apical spine.	1 apical spine.	1 apical spine.
	1 apical seta.	1 apical seta.	1 apical seta.
2 inner setæ.	3 inner setæ.	3 inner setæ.	3 inner setæ.

Third Foot.		Fourth Foot.	
Outer Ramus.	*Inner Ramus.*	*Outer Ramus.*	*Inner Ramus.*
Like second.		2 outer spines.	1 outer seta.
		1 apical spine.	
		1 apical seta.	2 apical spines (equal).
		3 inner setæ.	2 inner spines.

4. Two-jointed fifth foot.
5. The basal joint short and broad with a single seta on the outer margin.

A long, cylindrical, distal segment with a blunt, inner spine and a long, outer seta, but very slightly plumose.

6. The abdomen is composed of segments, the first of which is as long as the remaining segments combined. The caudal stylets are long.
7. The receptaculum seminis is broadly oval.
8. There are four hairs on the distal segment of the larger branch of the maxillipeds. The second segment has a large, immovable dactyl with a row of teeth along the edge, and with a

small hair at its base. Attached to the immovable dactyl is a small, movable one.

9. The antennæ are about the length of the cephalothorax.

The points in which the Long Island *Cyclops* that I have studied differs from *C. parcus* are: In the number of antennal segments, there being 13 instead of 17, and in the occasional variation in the armature of the outer ramus of the fourth foot, there being but one outer spine and one seta, where *C. parcus* has typically two spines; as well as in the armature of the terminal joint of the large ramus of the maxilliped, where two small hairs replace one large one; also in the armature of the distal joint of the fifth foot, which carries an outer hair, in place of the unserrated spine which is present in the form with seventeen joints in the antennæ.

As the correlated characteristics of species occur with great regularity in the *Cyclops*, and as the form under consideration seemed, both on account of its relatively large size and its fertility, to be a mature form, I searched for similar individuals but for a long time failed to find them.

In the summer of 1899, I had the opportunity of collecting large numbers of *Cyclops* at Cold Spring Harbor, L. I., where several fresh-water ponds afford excellent opportunities for the study of a variety of species. Though I have worked over some of this material with great care, I have never met with a single instance of a thirteen-jointed antenna.

In March of the present year, 1903, I again met with a number of *Cyclops* having thirteen-jointed antennæ. This material was collected in one of the large, shallow, fresh-water ponds at Jamaica, Long Island. The copepods were found in great numbers hidden beneath the fallen leaves along the edges of the pond. Again I noticed marked color-changes incident to the development and laying of ova. Some were red in the body and carried blue eggs in their paired sacs, while many were dark in color and carried about the partly developed reddish embryos.

Associated with these larger forms were smaller *Cyclops*, often without eggs, and emerald green to the naked eye, owing to the numbers of green protozoa that had attached themselves to the cuticle and almost concealed the host. The larger *Cyclops* with the pink bodies and the blue eggs, or *vice versa*, were comparatively free from the one-celled forms. I believe this fact is important as pointing to the strong probability of a recent moult. Further study revealed the fact that the larger forms had invariably seventeen segments in the antennæ and that they agreed in all essential details with the species known as *C. parcus* (Herrick).

After formulating data gathered from the study of species-characters in many different individuals from the same locality, I was able to clearly distinguish three groups, in all of which, all the leading species-characteristics of *C. parcus* (Herrick) were combined with a varying number of segments in the antennæ, which, however, all belonged to the same type (Fig. 1).

Group I. comprised individuals with thirteen antennal segments.

Group II. comprised individuals with fourteen antennal segments.

Group III. comprised individuals with seventeen antennal ments.

Nearly all of the *Cyclops* referred to as covered by protozoa and hence appearing green, belong to Group II. or are intermediate between Groups I. and II., and are characterized by antennæ with fourteen segments either fully formed, or in process of forming. I have studied no less than ten individuals which show clearly that the fourteen-jointed antenna is derived from the thirteen-jointed one, by the division of the tenth segment — the fourth from the distal end of the antenna — which is divided almost equally into halves by a transverse partition.

It is *always* the tenth segment which is dividing at this stage, and in all cases recorded, when the two antennæ are not in the same stage of division, it is without exception the *left* that is in advance of the right, in which division can still be seen in progress, as in Fig. 1, *B*.

I know of no explanation of the retarded division in the right antenna, and it may be a mere coincidence that all of my observations agree on this point. One *Cyclops* in which the fourteen segments were perfectly formed in both antennæ, proved particularly interesting, for I believe it furnishes positive proof

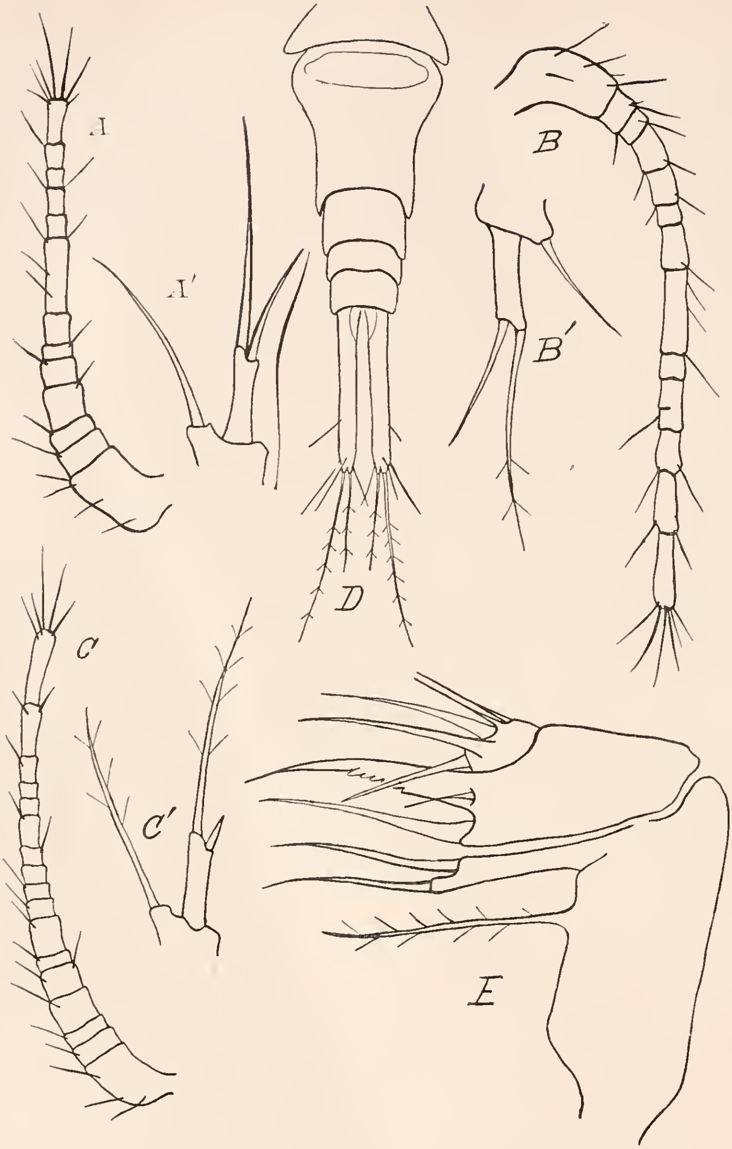

FIG. 1. Shows the antenna and fifth foot of a cyclops with thirteen-jointed antennæ, *A*, *A′*, Group I. The antenna and fifth foot of a cyclops with fourteen jointed antennæ, *B*, *B′*, Group II. The antenna and fifth foot of a cyclops with seventeen-jointed antennæ, *C*, *C′*, Group III. *D* shows the abdomen, the receptaculum seminis and the caudal stylets characteristic of all the forms with thirteen, fourteen or seventeen-jointed antennæ. *E* shows the large ramus of the maxilliped characteristic of the three forms. *B* also shows the tenth antennal segment in the act of dividing, thus giving rise to the fourteen-jointed antenna.

As compared with the length of the cephalothorax all the antennæ *A*, *B* and *C* shown in Fig. 1 are relatively long, extending to the first segment irrespective of the number of segments they contain.

that this apparently stable individual with the fourteen-jointed antennæ represents but a temporary condition in the development of a form with seventeen antennal segments. In the case referred to, the long eighth joint, that is characterized by three rather widely separated setæ, showed distinct, transverse lines across the segment at the level of each of the two lateral setæ. Half way up the remaining section, a slight indentation in the cuticle marked the position of the wall that completes the separation of this long segment into four small ones of almost equal size.

The breaking up of the eighth segment in the manner indicated by these markings gives to the seventeen-jointed antenna a short eighth segment with a single distal seta; a short ninth segment with a distal seta; a short tenth segment without any armature, and a short eleventh segment with one distal seta. These are precisely the conditions which prevail in the seventeen-jointed antennæ.

In his report on "The Entomostraca of Minnesota," Herrick describes a *Cyclops* strikingly like the one from Jamaica, Long Island, *with fourteen-jointed antennæ, three-jointed rami with the armature of the last joints like that given for C. parcus, and with ā two-jointed fifth foot* "with the armature like *C. strenuus*, which also resembles *C. pulchellus*." The stylets are very long. These correlated peculiarities of structure are recognized as constituting a distinct species known as *Cyclops insignis* (Claus). Herrick mentions that "in a previous edition it was suggested that this is but an atavistic form of *C. pulchellus* — *C. strenuus*." If *C. strenuus* is to be regarded as practically the same form as *C. abyssorum*, as Schmeil suggests, the Long Island form can hardly be brought into relation with it, for the armature of the swimming feet, which is remarkably constant in forms of equal size, differs markedly in the two cases. Schmeil, however, seems to attach little importance to this fact.

That the Long Island form with the fourteen-jointed antennæ represents a transitional stage in the development of a seventeen-jointed form, there can be little doubt, though the determining of the species in the terms of an old and confused classification is by no means an easy matter. The length of the caudal stylets is relatively greater in the *Cyclops* with the fourteen-jointed antenna than in the adult *C. parcus*, though in *C. parcus* the stylets are characteristically long. Another slight difference is seen in the presence of a hair in the form with the fourteen-jointed antennæ, in place of a small spine on the inner angle of the distal joint of the fifth foot of the seventeen-jointed form. Compare *A′*, *B′*, *C′*, Fig. 1. Moreover, the distal joint of the so-called *C. insignis* is strikingly long, longer than the corresponding joint in *C. parcus*. The difference between the two forms seems to be almost entirely one of proportion and size, the *insignis*-like form being slightly smaller than *C. parcus*, and often with fewer or no eggs.

In favor of the existence of a separate species for those forms with fourteen-jointed antennæ, and against the suggestion made by Herrick that *C. insignis* represents a transitional stage in development, Schmeil urged the occurrence of the *Cyclops* in large numbers, and its relatively large size, both of which *observations* I can confirm. I can not, however, agree with Schmeil's interpretation ; although the form is abundant and moderately large, it is often, though not always, without eggs either in the body or attached, when older forms associated with it are remarkably prolific. Moreover, if studied at the right stage, the form with fourteen segments in the antennæ gives frequent signs of being still in a period of growth characterized by morphological changes. The fact that the smaller form is densely covered by foreign growths indicates that it has not very recently moulted. In this connection it may not be irrelevant to allude to a few observations made on isolated copepods.

I separated a number of *Cyclops* in a small watch crystal. All were about the same size, some green to the naked eye, some dark, and others carrying eggs. A few days later my attention was drawn to a bright red *Cyclops* with a perfectly clean cuticle. It had seventeen segments in the antennæ, and from the absence of protozoa on its surface it must have moulted quite recently. I then looked about in the dish for cast-off skins and found one still well covered with protozoa and having fourteen-jointed antennæ.[1]

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[1] Inasmuch as there were other individuals in the watch crystal, this is by no means *conclusive* proof that the seventeen-jointed form had shed the fourteen-jointed skin, but I could find no other explanation of its presence in the dish and I offer the fact for whatever it is worth.

I then set aside six *Cyclops* with fourteen-jointed antennæ, giving them clean hydrant water containing but little food and some fresh-water plants. At the time of their separation two had fourteen segments only in the left antenna, while the right antenna of each contained a dividing segment, the tenth from the base of the antenna, or the fourth from the distal end. Two weeks later the division of the segment was still incomplete, showing that in this case at least, the formation of partition walls is not very rapid. The bodies looked lighter and clearer than before, and I examined them again to see if any changes had taken place, but none had occurred.

In his explanatory notes accompanying Plate XXXIV.[1] which shows the species-characteristics of *C. parcus* (Herrick), Herrick shows "caudal stylets of an elongate form," in Fig. 3, with which my own drawings agree perfectly. It is quite possible that the elongated distal segment of the fifth foot may be a mere variation correlated with the elongation of the caudal stylets in Herrick's 'elongated form' of *C. parcus* which he suggests "is to be regarded as a post-imago."

A single characteristic which Herrick describes for *C. pulchellus*, but of which no mention is made in the characterization of *C. parcus*, to my knowledge, is the presence of serrations on the distal margins (ventral and lateral) of the last abdominal segment, while the remaining margins of the abdominal segments are free from such markings. All of the individuals of the three groups — *i. e.*, of the thirteen-, the fourteen- and the seventeen-jointed antennæ — agree with *C. pulchellus* in having these serrations, while Groups I. and II. also agree in having "two rather long setæ" which are not at all or only slightly plumose on the terminal segment of the fifth foot. But they all differ from *C. pulchellus* in not having the basal joint of the fifth foot longer than wide; the basal joint is unequivocally wider than it is long, and in this respect agrees with *C. parcus*.

Although the armature of the appendages is very constant in the Cyclopidæ, it is quite common to meet with similarly placed spines and setæ of different lengths. A notable instance of this occurs in the armature of the third joint of the large ramus of the maxillipeds of the fourteen-jointed and seventeen-jointed forms. The armature usually consists of three large hairs and two very small ones growing close together at the base of one of the large hairs (Fig 1, *E*). In the fourteen-jointed forms, these two small hairs are strikingly shorter than they are in the seventeen-jointed form. With this single exception, the maxillipeds are precisely alike in both groups.

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[1] "Copepoda, Cladocera and Ostracoda of Minnesota," Zoölogical Series, II., 1895, of the Geological and Natural History Survey of Minnesota.

I am aware that Herrick describes the armature of the terminal segment of the larger branch of the maxilliped of *C. parcus*, as consisting of four hairs. I have found an instance in which four large hairs of almost uniform size occur, but a more frequent condition in the Long Island *Cyclops* is seen in those instances which show three large hairs and two short ones, in place of the four hairs of Herrick (Fig. 1, *E*).

Among the many *Cyclops* I have studied, I have seen but one with eighteen segments in the antennæ. In this case the eighteenth segment is derived from the seventh segment, by transverse division, at the level of the seta. In both right and left antennæ the division is incomplete, extending but half way across the segment.

I have studied this *Cyclops* with great care, and in every detail of structure, it agrees perfectly with the forms associated with it in showing the chief species-characteristics of *C. parcus.*

I have repeatedly made written records of body-segments and appendages showing the complete armatures, and have made many outline drawings of those parts that are correlated in the determination of species, and I believe no room for doubt remains that the *Cyclops* with thirteen and with fourteen antennal segments, as well as the form with eighteen segments, are all to be referred to the type with seventeen segments in the antennæ. Those having thirteen and fourteen segments, known as *C. insignis*, though very abundant forms and though sexually mature, do not represent a group of sufficient permanency to warrant us in regarding them as representatives of a distinct species. They are rather to be considered as transitory stages which, though capable of producing young, have not as yet attained their maximum growth, or their highest degree of complexity.

The *Cyclops* with the eighteen-jointed antennæ agrees with Claus' description of *Cyclops elongatus*, so far as Herrick has quoted Claus. Nevertheless, its close agreement in all species-characteristics with *C. parcus*, with which it was found, and the very exceptional occurrence of so many antennal segments, make it highly probable that we are dealing here with a case of variation rather than with a species-character.

The *Cyclops* from Cold Spring Harbor, Long Island, were collected at the surface of a very shallow pond along a road-side near the laboratory of the Brooklyn Institute. The pond was choked with water-plants and a scum of duck-weed floated on the surface. From the extreme shallowness of the pond, any life there must have been exposed to rapidly changing conditions. The material collected in this pond was all taken from one locality within a radius of a few feet, where the copepods were in among the duck-weed.

I attempted some statistical studies in variation on these forms, but the work was soon interrupted by the comparatively small number of individuals belonging to the same species, or to species closely enough related to warrant any use of them in obtaining data. Most of the forms I have been wholly unable to identify, for while they agree with well known species in certain characteristics, they differ from them in others which are apparently no less important.

Certain combinations of characters occur so frequently, that, in the absence of transitional forms, one is often tempted to believe that in the bewildering array of forms before him, he is dealing with new variations, of which it is almost impossible to say whether they have a species value or not. Whether the forms met with illustrate pædogenesis, or whether the season was connected in any way with the morphological aspect of the copepods, I cannot say, not having been able to collect from this vicinity at any other season. But I have not seen any transitional stages in an individual such as would warrant the linking of it with any well known species.

One *Cyclops* frequently met with, combines the following characteristics: *Antennæ nine-jointed; rami of swimming feet two-jointed; rudimentary fifth foot one-jointed.*

Armature of the Swimming Feet.

First Foot.		Second Foot.	
Outer Ramus.	*Inner Ramus.*	*Outer Ramus.*	*Inner Ramus.*
3 outer spines.	1 outer seta.	3 outer spines.	1 outer seta.
1 apical spine.	1 apical spine.	1 apical spine.	1 apical spine.
1 apical seta.	1 apical seta.	1 apical seta.	1 apical seta.
4 inner setæ.	5 inner setæ.	4 inner setæ.	5 inner setæ.

Third Foot.		Fourth Foot.	
Outer Ramus.	*Inner Ramus.*	*Outer Ramus.*	*Inner Ramus.*
3 outer spines.	1 outer seta.	3 outer spines.	1 outer seta.
1 apical spine.	1 apical spine.	1 apical spine.	2 apical spines.
1 apical seta.	1 apical seta.	1 apical seta.	
4 inner setæ.	4 inner setæ.	4 inner setæ.	3 inner setæ.

The antenna and fifth foot of this form are seen in Fig. 2.

The pravalence of the form alone is not sufficient reason for regarding it as a distinct species, and the probability is that we are here dealing with a transitional stage in the development of a species with a greater number of antennal segments, as seen in the case of the fourteen-jointed form, for no species in its mature condition is recognized as having nine antennal segments, while the fact that *the rami are two-jointed and the number of setæ on the last joint of the inner ramus is exceptionally large*, suggests that the rami may subsequently acquire a third joint. Moreover, the armature of the feet is strikingly like the armature of another *Cyclops* having ten antennal segments.

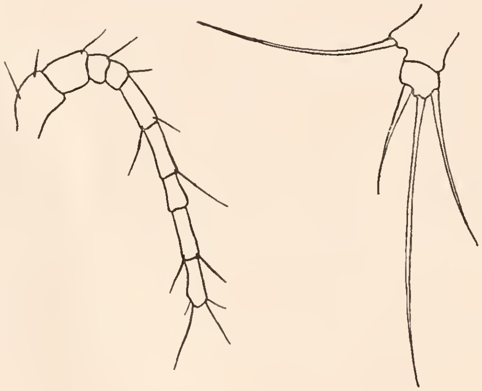

Fig. 2. Shows the antenna and the fifth foot of a cyclops with nine antennal segments. The fifth foot is two-jointed and resembles the fifth foot of the cyclops with the ten-jointed antennæ.

This second form which occurs frequently in the same locality, combines the following characteristics: *Antennæ ten-jointed; rami of swimming feet two-jointed; rudimentary fifth foot two-jointed.*

Armature of the Swimming Feet.

First Foot.		Second Foot.	
Outer Ramus.	*Inner Ramus.*	*Outer Ramus.*	*Inner Ramus.*
3 outer spines.	1 outer seta.	3 outer spines.	1 outer seta.
2 apical spines.	1 apical spine.	1 apical spine.	1 apical spine.
3 inner setæ.	1 apical seta.	1 apical seta.	1 apical seta.
	5 inner setæ.	4 inner setæ.	5 inner setæ.

Third Foot.		Fourth Foot.	
Outer Ramus.	*Inner Ramus.*	*Outer Ramus.*	*Inner Ramus.*
3 outer spines.	1 outer seta.	3 outer spines.	1 outer seta.
1 apical spine.	1 apical spine.	1 apical spine.	1 apical spine.
1 apical seta.	1 apical seta.	1 apical seta.	1 apical seta.
4 inner setæ.	4 inner setæ.	4 inner setæ.	3 inner setæ.

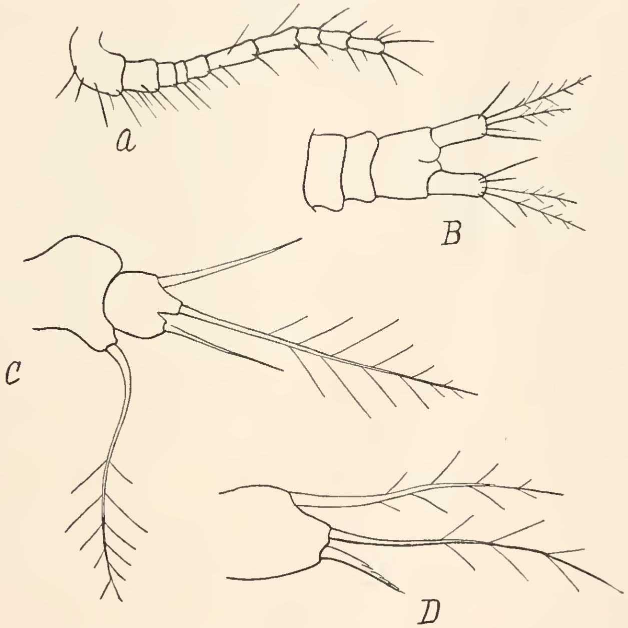

Fig. 3. The antennæ, the abdomen and caudal stylets and the two types of fifth foot correlated with the 10-jointed antennæ. *C* shows the fifth foot correlated also with the 9-jointed antennæ, while *D* shows the fifth foot correlated with the 11-jointed antennæ. *B* represents the type of abdomen and stylets correlated with 10 antennal segments irrespective of the form of the fifth foot.

Wherever these forms with the nine- and ten-jointed antennæ occur they show the same striking similarity in the armature of the swimming feet. *The nine-jointed forms are perfectly constant* *throughout the group*, but *the ten-jointed forms vary considerably within the group*, occasionally combining three-jointed rami with a two-jointed fifth foot, and occasionally two-jointed rami with a one-jointed fifth foot.

According to Herrick's classification of the Cyclopidæ, there is but one species having ten-jointed antennæ, *i. e.*, *C. phaleratus*, which may combine either *ten- or eleven-, usually eleven-jointed, antennae with three-jointed rami in the swimming feet, and with a one-jointed fifth foot.* I have found this combination in a single case, and the antennæ contained each eleven segments. Herrick gives only the formula for the fourth foot of *C. phaleratus*, with which the above form also agrees. The entire armature of the terminal joints of the four swimming feet in the Cold Spring Harbor form is shown below.

First Foot.		Second Foot.	
Outer Ramus.	*Inner Ramus.*	*Outer Ramus.*	*Inner Ramus.*
3 outer spines.	1 outer seta.	3 outer spines.	1 outer seta.
1 apical spine.	1 apical spine.	1 apical spine.	1 apical spine.
1 apical seta.	1 apical seta.	1 apical seta.	1 apical seta.
3 inner setæ.	3 inner setæ.	4 inner setæ.	3 inner setæ.

Third Foot.		Fourth Foot.	
Outer Ramus.	*Inner Ramus.*	*Outer Ramus.*	*Inner Ramus*
3 outer spines.	1 outer seta.	2 outer spines.	1 outer seta.
1 apical spine.	1 apical spine.	1 apical spine.	2 apical spines.
1 apical seta.	1 apical spine.	1 apical seta.	
4 inner setæ.	3 inner setæ.	4 inner setæ.	2 inner setæ.

The length of the antenna in *C. phaleratus* as compared with the cephalothorax is short, whereas in the Cold Spring Harbor form the antennæ are relatively long, extending nearly to the second thoracic segment. Moreover, in a single instance the long second joint of the antenna showed a light, transverse band near its proximal margin, suggesting the characteristically short second segment of the eleven-jointed antenna.

The chief characteristics of the cyclops with the eleven-jointed antennæ are *three-jointed rami in the swimming feet combined with a two-jointed fifth foot* (Fig. 4).

Herrick recognizes three species having eleven antennal segments; one of these is a European form of marked peculiarity; a second is *C. diaphranus*, whose species-characteristics are *eleven-jointed antennæ, two-jointed rami in the swimming feet, and a one-jointed fifth foot, with a long seta and one short spine.*

I have not found a single *Cyclops* combining these characters. The eleven-jointed antennæ are, with one exception, so far as my studies show, always correlated with *three*-jointed rami in the swimming feet, and the armature of these forms is precisely like that of *C. phaleratus*, whether the fifth foot be one-jointed or two-jointed. The third species having eleven-jointed antennæ which

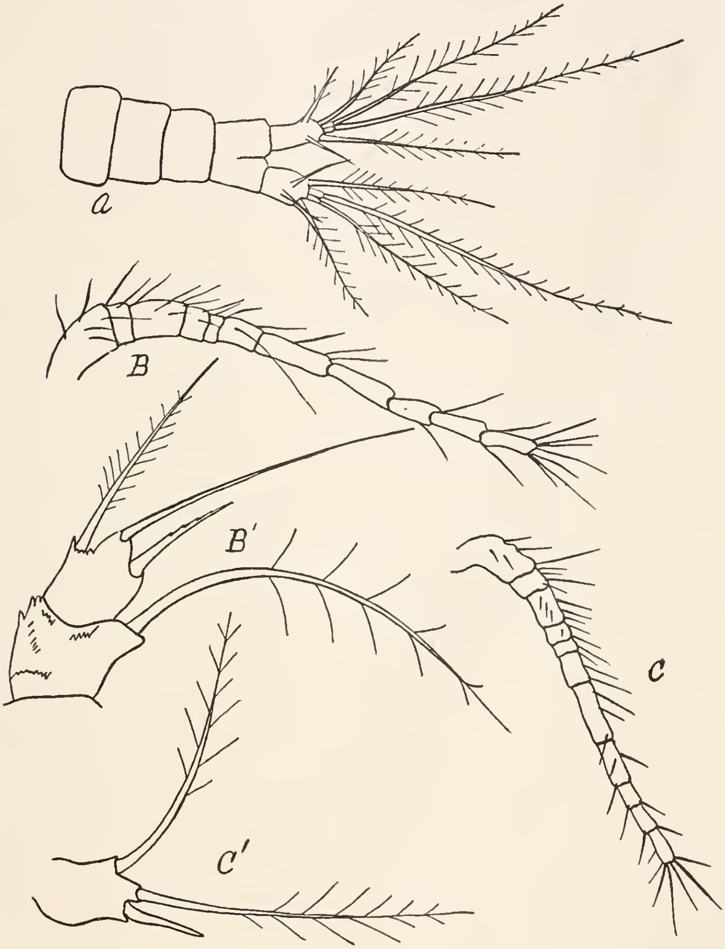

Fig. 4. Represents an eleven-jointed antenna, *B*, correlated with a two-jointed fifth foot *B′* and short caudal stylets with very long, plumose setæ, *A*. *C* and *C′* represent an eleven-jointed antenna and a correlated one-jointed fifth foot with the same abdomen and stylets as are seen in the form with the eleven-jointed antennæ and the two-jointed fifth foot.

Herrick recognizes, also combines a one-jointed fifth foot with two-jointed rami. It is known as *C. affinis* and is like *C. phaleratus*, "which it closely resembles."

A fourth and a last type to which I shall refer, is seen in a not infrequently occurring form *which combines twelve-jointed antennæ with three-jointed rami in the swimming feet and a two-jointed fifth foot* (Fig. 5).

Herrick recognizes three species as having these characteristics, namely: *C. capillatus* and *C. crassicaudis*, both European forms, and *C. varicans*, an American form. The two former are described as Scandinavian forms only. Of the third species *C. varicans*, Herrick says that it is "the American species most nearly resembling the European form with twelve antennal segments and a two-jointed fifth foot." "Unhappily," Herrick also remarks, "this species was taken but once." On Plate XXX.[1] Herrick figures the first foot of *C. varicans*, which he pictures as having two-jointed rami in the swimming feet. Herrick explains that the last joint is homologous to two fused segments, and that the separation might take place "at the next moult." The form I have studied shows the armature when the rami have reached

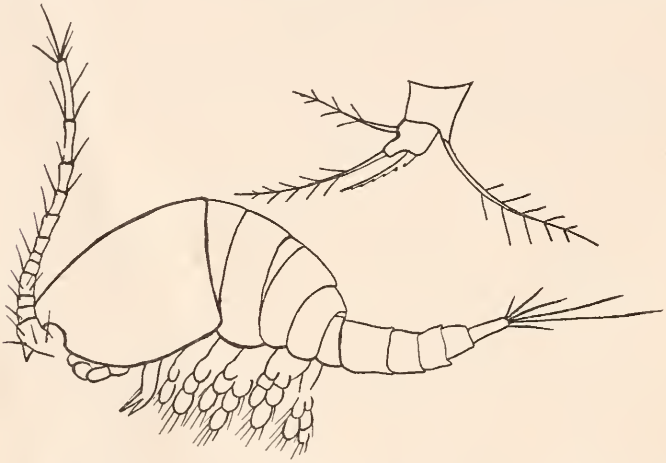

FIG. 5. Shows a twelve-jointed antenna which is relatively very long as compared with the cephalothorax, notwithstanding the relatively small number of antennal segments present.

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[1] "Copepoda, Cladocera and Ostracoda of Minnesota."

the three-jointed condition, and the reduction in the number of spines and setæ in the armature of the fourth foot might seem to bear out Herrick's suggestion.

C. Varicans. Fourth Foot.		Cold Spring Harbor Cyclops. Fourth Foot.	
Outer Ramus.	*Inner Ramus.*	*Outer Ramus.*	*Inner Ramus.*
3 outer spines.	1 outer seta.	2 outer spines.	1 outer seta.
1 apical spine.	1 apical spine.	1 apical spine.	2 apical spines.
1 apical seta.	1 apical seta.	1 apical seta.	
4 inner setæ.	4 inner setæ.	4 inner setæ.	2 inner setæ.

The armature of the Long Island form suggests *C. phaleratus*, though in the first foot it is not identical.

I have found three of these forms among a relatively small number of individuals and they agree very closely with one another, the only difference being in a slight variation in the armature of the swimming feet, a spine occasionally appearing in place of a seta.

Supposing that these individuals represent *C. varicans*, the Cold Spring Harbor form is very evidently in a later stage of development than the individual figured by Herrick. Any appeal to relative ages as an explanation of differences, requires the supposition that some of the segments of the feet have an adult armature while other segments have not. But there is no reason for supposing that the number of spines and setæ in the fourth foot is incident to the breaking up of the rami into three segments instead of two, for the armature of the first foot is not reduced by the presence of the additional joint in the rami.

Summary.

The Long Island *Cyclops* (*C. insignis?*), having fourteen-jointed antennæ, three-jointed rami in the swimming feet, with two-jointed fifth feet and elongate caudal stylets, is a transitional stage in the development of a seventeen-jointed form *C. parcus* (Herrick ?). The eighteen-jointed antenna is derived from the seventeen-jointed form by division of the seventh segment.

Out of fifteen individuals taken at random, none of whose antennal segments exceed twelve, five precisely similar individuals constitute a group having *nine antennal segments*, *two-jointed rami* and *two-jointed fifth feet.*

Four individuals constitute a second group having typically *ten antennal segments*, *two-jointed rami*, and *two-jointed fifth feet.* Two of these individuals show marked variation, one in having *three-jointed rami* in the swimming feet, the other in having a *one-jointed fifth foot.*

Four individuals constitute a third group, characterized by *eleven-jointed antennæ*, *three-jointed rami*, and *two-jointed fifth feet.* One member of this group has a *one-jointed fifth foot*, and *this is the only individual out of the thirteen that can be given any place among species, i. e., C. phaleratus*, as combining well recognized species-characters.

Three individuals constituting a fourth group combine the following characteristics: *twelve-jointed antennæ*, *three-jointed rami*, and *two-jointed fifth feet.* These forms suggest *C. varicans*, with which they have much in common, but from which they differ considerably in detail.

Some facts point to the probability that the Cold Spring Harbor forms with the ten-jointed antennæ are morphologically undeveloped. Especially does the variation within the group consisting of but few individuals point to the instability of these forms.

What the true nature of these correlated peculiarities in *Cyclops* may be, can only be determined by following the life history of each individual. The relatively large size of these forms, and the frequency with which they occur, as well as the constancy of the correlated characteristics, suggest on first acquaintance with the Cyclopidæ, that they represent distinct species, but a fuller acquaintance warns us to look further for an explanation of these most perplexing variations which are doubtless largely due to the acquiring of sexual maturity while the morphological changes in the body are still incomplete, and to the varying external conditions to which they are subjected.

BROOKLYN, NEW YORK,
March 30, 1903.

ON THE OCCURRENCE AMONG ECHINODERMS OF LARVÆ WITH CILIA ARRANGED IN TRANSVERSE RINGS, WITH A SUGGESTION AS TO THEIR SIGNIFICANCE.

CASWELL GRAVE.

In this paper a short account is given of some observations made at the laboratories of the United States Fish Commission at Woods Hole and Beaufort on the larvæ of various echinoderms. The attempt is also made to show that these observations, taken together with those made by other students of the group, have a direct bearing upon one phase of the problem of the early ancestry of the echinoderms.

It would be quite impossible to give an intelligible discussion of the bearing these observations are interpreted to have upon this subject without first recalling the hypotheses which have been put forward by other students of the group to account for its origin and present organization.

The hypothesis which now seems to have the most general acceptance is not the work of any one mind but represents the work of many. It would be difficult, therefore, in giving a hasty review of its most important points, to credit each of its authors with just his contribution, so I shall make only such comments in passing as will serve to explain the changes and additions which seem to me to be warranted.

Observations.

Holothurians.

The barrel-shaped pupæ of Holothurians have been long known, having been described by Müller,[1] Semon[2] and others. They arise in each case by the breaking up and rearrangement of the ciliated bands of the fully formed auricularian larvæ at the time when the metamorphosis into the adult form is about to take place. Semon's figures of the auricularian larva and the pupa of *Synapta digitata* are reproduced in outline in Figs. 1 and 2. During the pupal stage the mouth shifts from a ventral to a terminal position and the tentacles and tube feet first become functional. The ciliated rings of the pupa are five in number and are arranged transversely to its long axis.

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[1] J. Müller, "Abhandlungen über die Larven und Metamorphose der Echinodermen," *Abl. Kgl. Akad. Wiss. Berlin.*

[2] R. Semon, "Die Entwicklung der Synapta digitata, und die Stammesgeschichte der Echinodermen," *Jena Zeitschr.*, Vol. XXII., 1888.

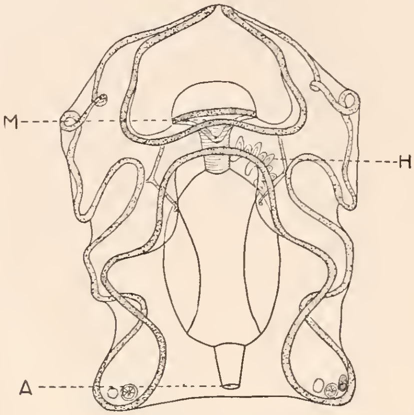

FIG. 1. Auricularian larva of *Synapta digitata*. After Semon. *M*, mouth; *H*, hydrocœle; *A*, anus. The ciliated bands stippled.

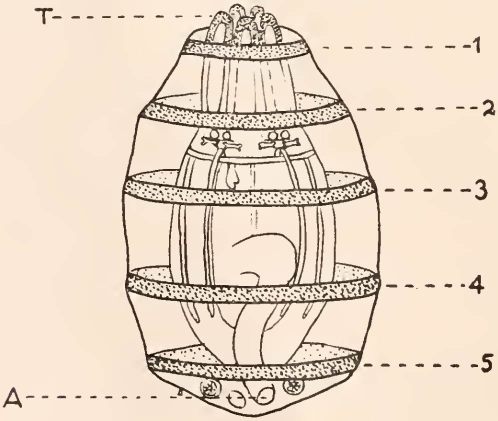

FIG. 2. Pupa of *Synapta digitata*. After Semon. *A*, anus; *T*, tentacles; 1, 2, 3, 4 and 5, ciliated rings.

Selenka[1] has studied and figured the larva of *Cucumaria doliolum* which, although totally unlike an auricularian larva, can be well compared with a pupa. It is an elongated free swimming creature with four, sometimes five, transversely arranged ciliated rings, in addition to which, at the anterior end, there is a ciliated field. This ciliated field is one of the first of the larval structures to disappear as development progresses. In Selenka's figure of this larva, reproduced in outline in Fig. 3, five tentacles and two tube feet are shown to be developed and the rotation of the mouth and tentacles to the terminal position has begun. The eggs of *C. doliolum* are quite large and well supplied with yolk,

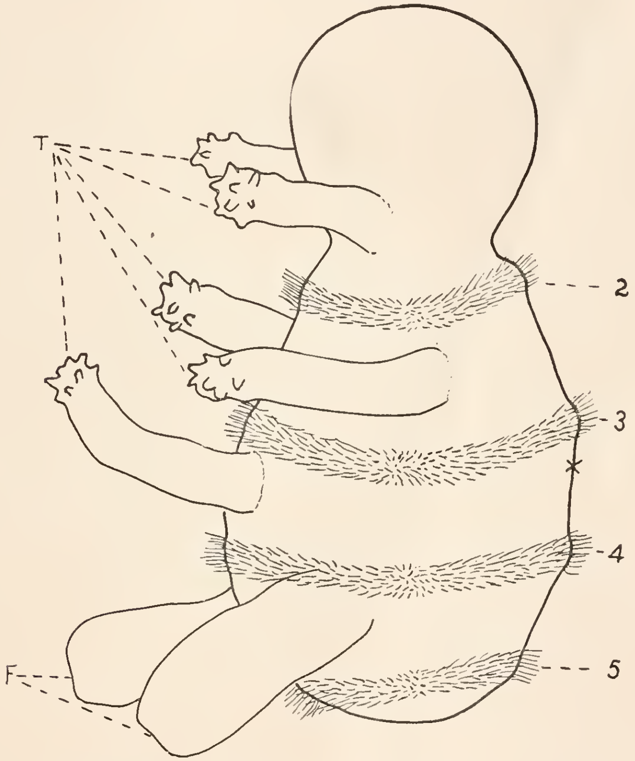

FIG. 3. Larva of *Cucumaria doliolum*. After Selenka. *F*, tube feet; *T*, tentacles; 2, 3, 4 and 5, ciliated rings.

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[1] E. Selenka, "Zur Entwicklung der Holothurien," *Zeit. f. wiss. Zoöl.*, Vol. XXVII., 1876.

thus differing widely from the small transparent eggs of *Synapta digitata*. The efficient locomotor and feeding apparatuses with which the larva of the latter species is provided, enabling it to care for itself, are not needed by the larva of *Cucumaria doliolum* for whose care provision has already been made. The larva of *Cucumaria* can, as it were, give its whole attention to the production of a creature with the structure of the adult while the larva of *Synapta* must make this secondary to food getting.

Crinoids.

In *Antedon rosacea*, the only species of crinoid the development of which has been studied, the eggs are supplied with considerable yolk and for a time the developing larvæ are brooded.

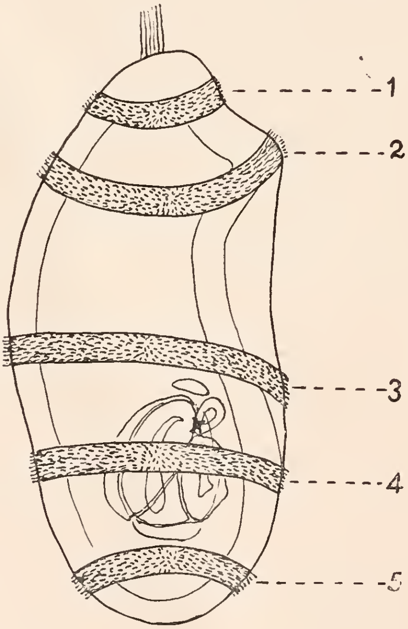

FIG. 4. Larva of *Antedon rosacea*. After Seeliger. Internal organs shown in posterior end. 1, 2, 3, 4 and 5, ciliated rings.

The free-swimming period is of short duration and the development is more or less direct. The larva is elongated and cylindrical and is encircled by five transverse ciliated rings. An apical tuft of longer cilia is also present. Seeliger's[1] figure of it is reproduced in outline in Fig. 4. No pore canal is developed at this stage but the point on the hydrocœle at which it will appear later, I have indicated by a small *x*.

Ophiurids.

For a long time the larvæ mentioned above were the only observed cases in which the ciliated bands are arranged in transverse rings, and they were considered to have no special significance. Since 1899, however, I have found three other cases which exhibit the same peculiarity and which represent two other classes of echinoderms.

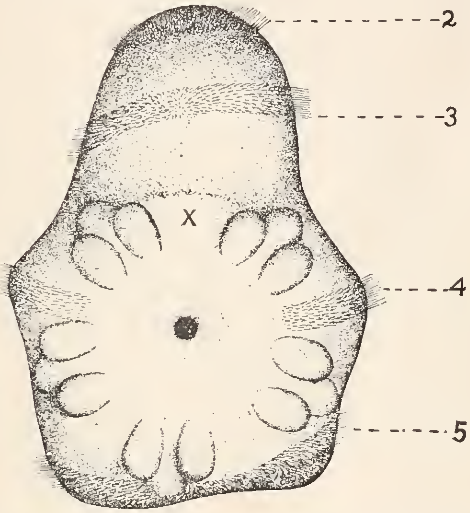

FIG. 5. Ventral view of the young larva of *Ophiura brevispina*. Original. 2, 3, 4 and 5, ciliated rings.

The larva of *Ophiura brevispina*, which I[2] described in 1899, is well supplied with yolk and very early in its development it sinks to the bottom and clings to grass blades where it undergoes its late larval stages and final metamorphosis. It is a larva without arms or processes of any kind and no skeletal rods such as are found in ophiuran plutei are developed, although at one time I mistook the beginnings of the skeletal plates of the adult for such. The anterior end of the larva is produced into a long preoral lobe about which two ciliated rings are developed. The posterior end is enlarged and contains the various internal structures of the larva and developing ophiurid. The mouth is ventral and interrupts the third ciliated ring of the larva (numbered 4 in Fig. 5). The fourth ring (5) surrounds the posterior end. The dorsal pore is situated at the point indicated by the small *x* between ciliated rings 3 and 4. As development progresses the preoral lobe diminishes in size until finally it is entirely absorbed. During late larval life a change in the arrangement of one of the ciliated rings also takes place. The ring numbered 5 becomes interrupted on the ventral side and takes on a more definite relation to the mouth (see Fig. 6).

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[1] O. Seeliger, "Studien zur Entwicklungsgeschichte der Crinoiden," *Zoöl. Jahrb.*, Bd. VI., 1892.

[2] Caswell Grave, "Ophiura brevispina," *Mem. Natl. Acad. Sci.*, 1900.

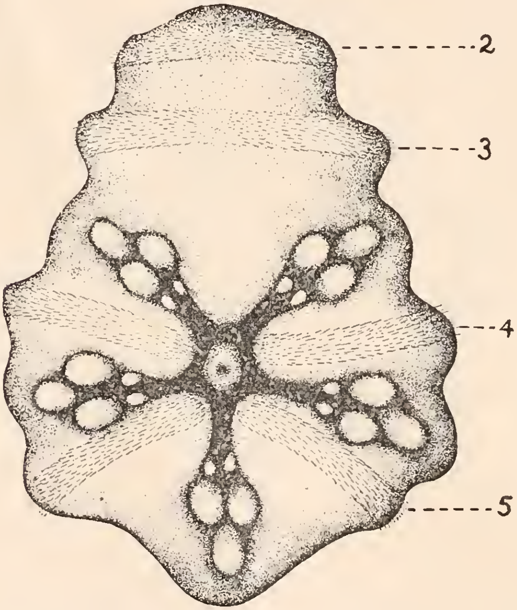

FIG. 6. Older larva of *Ophiura brevispina*. Original. The change in position which takes place in the ciliated ring (5) is shown.

In 1900 I found a second ophiuran larva at Beaufort which, in its metamorphosis from the pluteus to the radial form, showed the same tendency to rearrange the ciliated bands into transverse ciliated rings which is found among the holothurians. The outline of the pluteus is shown in Fig. 7. When the developing ophiurid has become quite large and the tissues of the pluteus are being absorbed, the ciliated bands of certain of the arms become applied to the disc in a quite definite manner, viz., about the madreporic interradius which had an anterior position in the larva, a complete ring is formed; an interrupted ring is laid down between rays 5 and 4 on one side and 1 and 2 on the other. A third ring crosses the base of ray 3. Not until I had examined a number of these metamorphosing plutei was I satisfied that this arrangement of the ciliated areas was not accidental but in all cases examined (a dozen or more) the arrangement was practically the same as that shown in Fig. 8.

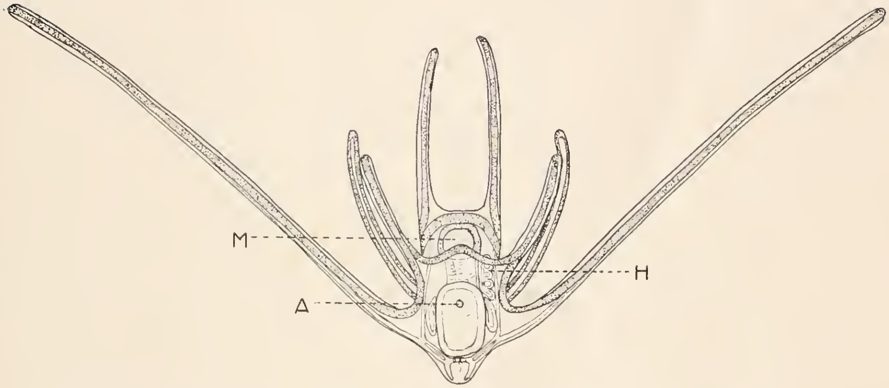

FIG. 7. Ophiuran pluteus (sp.?) from the "tow" at Beaufort. *A*, anus; *M*, mouth. *H*, hydrocœle. Ciliated bands stippled.

Echinoids.

During the summers of 1900, 1901 and 1902 I succeeded in rearing large broods of the larvæ of *Mellita testudinata* from the fertilized egg to the form in which the adult structure is attained. The larva is a typical highly specialized pluteus as will be seen from the outline of Fig. 9. The just metamorphosed *Mellitas* all showed three parallel transverse ciliated rings; the middle one of which is interrupted by the mouth (see Fig. 10). The function of these ciliated rings in the young *Mellitas* is probably to assist them in feeding until the tube feet have grown sufficiently to assume the function.

The Hypothetical Bilateral Ancestor.

Although numerous papers have been written on the subject of the phylogeny of the echinoderms there are but few which retain their vitality at the present time. In these, notwithstanding the fact that many differences in detail exist, there is a very great similarity in the views set forth and I may state in this connection that the facts of this paper and many of my unpublished observations are an additional support to the hypothesis which has been gradually developed by Bury, McBride and

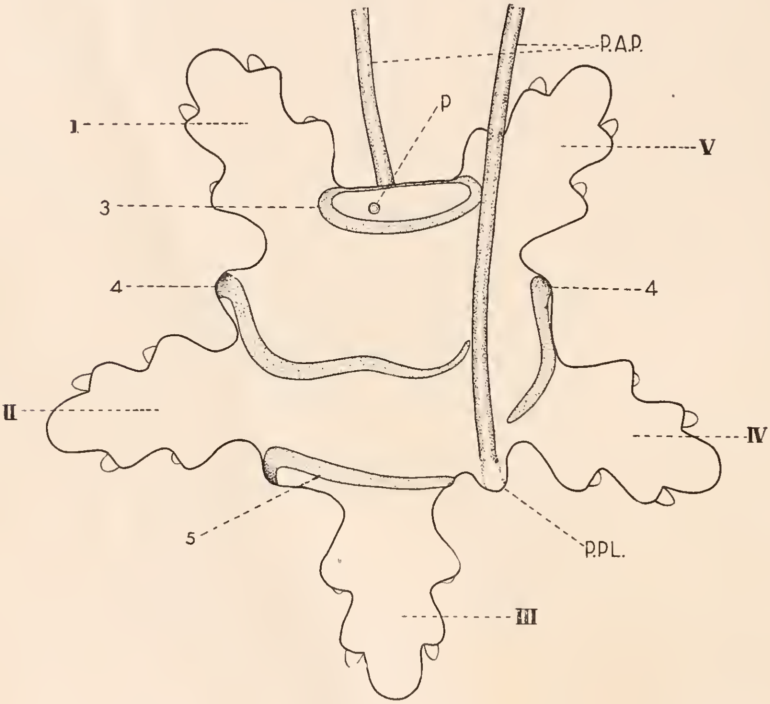

Fig. 8. Outline of the young ophiuran which metamorphoses from the pluteus shown in Fig. 7. Original. I., II., III., IV. and V., arms of the ophiuran; 3, 4 and 5, ciliated rings. *P. Pl.*, remnant of the posterior end of the pluteus. *P. A. P.*, long posterior arms of the pluteus which are never absorbed but are finally dropped. *P.*, madreporite.

Bather, and serve to carry it one step further. Each of these students has reconstructed the hypothetical ancestor both in its bilateral free swimming stage and the stage during which it became radially symmetrical. The same plan is followed in this paper.

The papers of Bury,[1] McBride[2] and Bather[3] in which the hypothetical bilateral ancestor of the echinoderms is reconstructed and figured, are so well known and the reasons for every detail of the anatomy of the creature are therein so well set forth that it would be a waste of the reader's time to again do more than give an outline of the supposed structure of the hypothetical organism, discussing such points only in which a change is made.

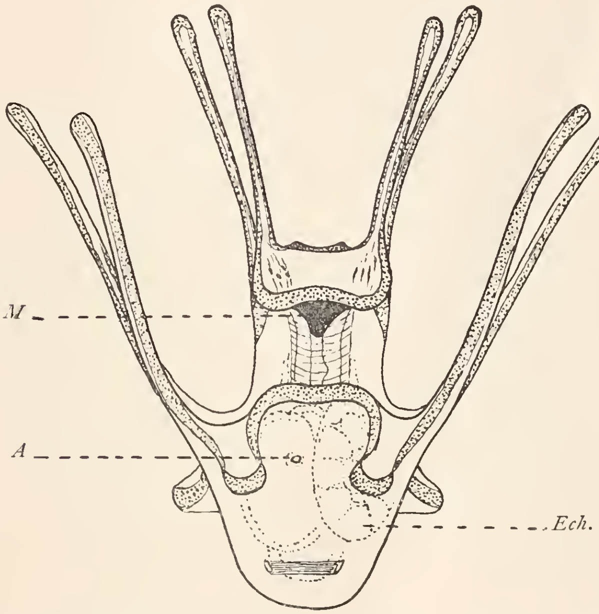

FIG. 9. Pluteus of *Mellita testudinata*. Original. *A*, anus; *Ech.*, developing sand dollar; *M*, mouth. Ciliated bands stippled.

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[1] Henry Bury, "The Metamorphosis of Echinoderms," Q. J. Mic. Sc., No. 149, 1895.

[2] E. W. McBride, "The Development of Asterina Gibbosa," Q. J. Mic. Sc., No. 151, 1896.

[3] F. A. Bather, "A Treatise on Zoology." Part III., "The Echinoderma." Edited by E. Ray Lankester, 1900.

Briefly then, the earliest ancestor of the group of echinoderms of which there is much trustworthy evidence, was a free-swimming organism of microscopic size with an elongated body and a long preoral lobe. At the tip of the preoral lobe a sense organ and nerve center was located. The contours of the body were plain, no arms or processes of any kind being present. The alimentary tract occupied the posterior part of the body, the mouth and anus opening ventrally. Three pairs of body cavities, arranged symmetrically with reference to the alimentary tract, were present.

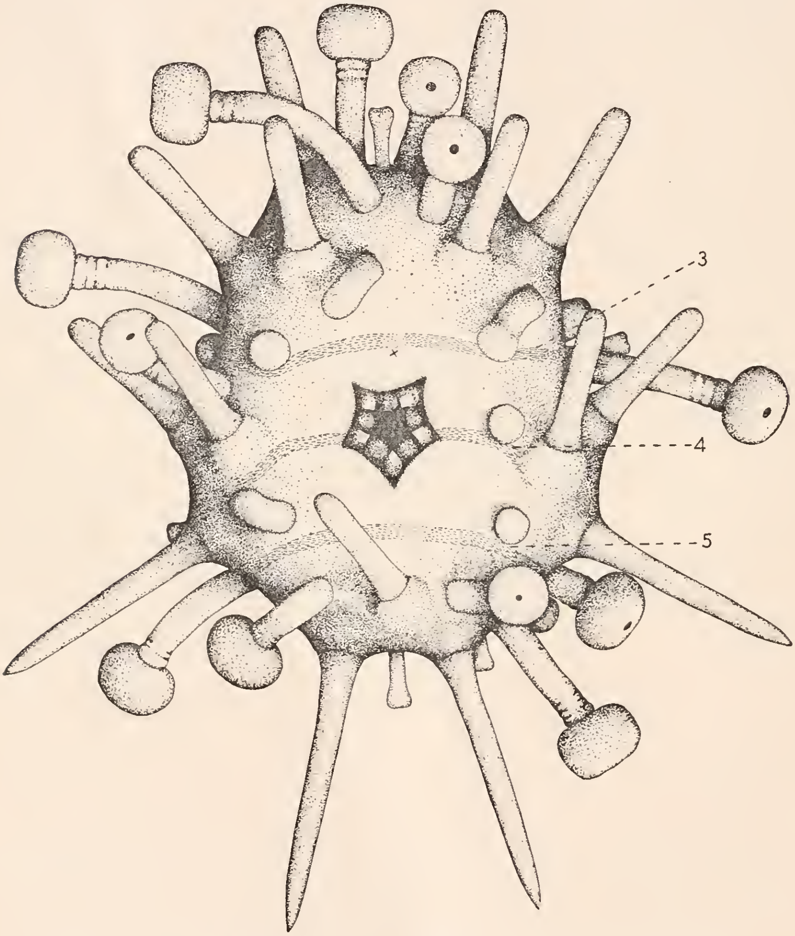


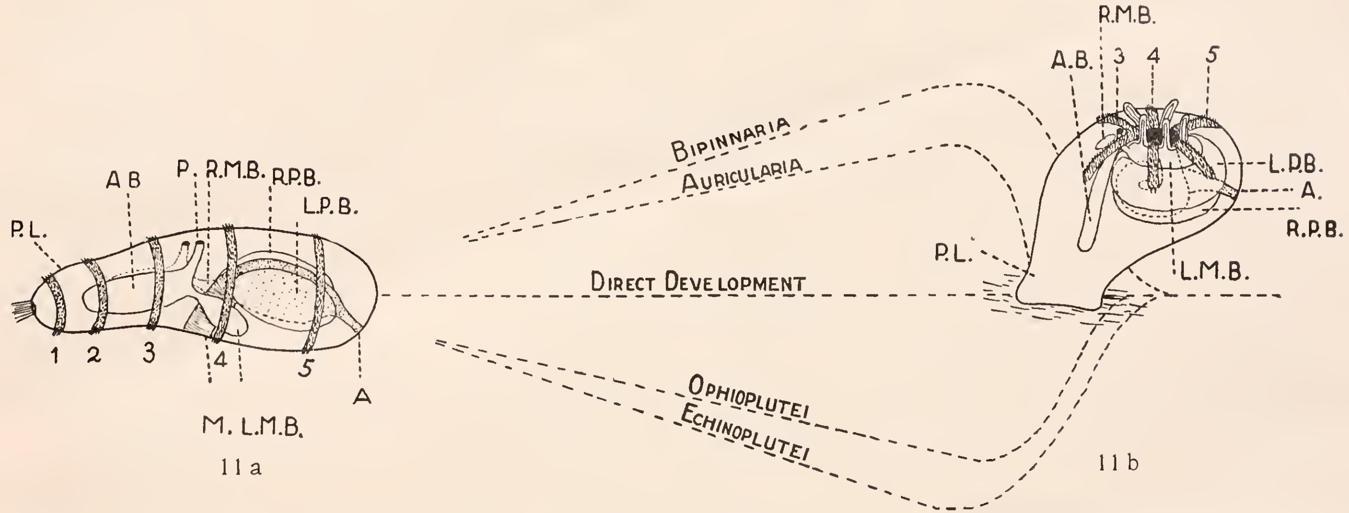
FIG. 10. Young *Mellita testudinata*. Original. 3, 4, and 5, ciliated rings.

The anterior pair extended into the preoral lobe where it may have been united into a single cavity. Posteriorly its cavities were placed on the right and left of the œsophagus and each cavity opened to the exterior, on the dorsal surface of the animal, through a ciliated duct. The posterior end of each anterior cavity was connected with the corresponding cavity of the middle pair by a second duct, also ciliated. The middle cavities were situated on either side of the point of union of the œsophagus and stomach. The posterior cavities were larger than those of the anterior and middle pairs and were applied to the stomach, forming a mesentery on the dorsal mid line.

If Fig. 11 *a*, of this paper is compared with Bury's Fig. 45, McBride's Fig. 157 and Bather's Fig. 1 it will be seen that the general plan is the same with differences in detail only.

Bury's idea that the hydrocœle (left middle body cavity) encircled the œsophagus (the right cavity having entirely disappeared) even during the period of the free-swimming existence of the animal, is, in the light of recent observations, an unnecessary assumption and one for which no explanation has been made. The changes which take place in the *posterior* pair of body cavities of echinoderm larvæ, by which the *left* one becomes horseshoe-shaped and encircles the stomach, are almost exactly similar to those by which the *left middle* body cavity takes on the form of a ring surrounding the œsophagus. If to explain the former it is necessary, as Bury and others believe, to assume a shifting of the position of the mouth and œsophagus incident to a life on the bottom, then a similar explanation for the latter is also called for. I agree with the more recent writers in the assumption that both the hydrocele and left posterior body cavity acquired their circular shape and position around the alimentary canal, at the same time, viz., during the period when the entire organization of the animal was being readjusted to its new conditions of life on the bottom.

According to McBride's hypothesis, each of the middle body cavities possessed, during the free swimming stage of the ancestor, five tentacles which were used in the capture of food. There is good evidence for the existence of two hydrocœles (middle body cavities), as McBride has shown in his work on


P.L.
A B
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Auricularia
Direct Development
Ophioplutei
Echinoplutei
R.M.B.
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L.M.B.
11 b

Fig. 11. Scheme to illustrate the suggestions of this paper concerning the struture and appearance of the hypothetical free-swimming and fixed ancestors of the echinoderms and the relation which the type of development of the more familiar echinoderm larvæ bears to the type which is represented by the larvæ of *Antedon*, *Cucumaria* and *Ophiura*. 11*a*, the hypothetical free-swimming ancestor. 11*b*, the hypothetical fixed ancestor. *A*, anus; *A.B.*, anterior body cavity; *L.M.B.*, left middle body cavity (hydrocœle); *L.P.B.*, left posterior body cavity; *M*, mouth; *P.*, dorsal pore; *P.L.*, preoral lobe; *R.M.B.*, right middle body cavity; *R.P.B.*, right posterior body cavity; 1, 2, 3, 4 and 5, ciliated rings.

Asterina, and as is further demonstrated in the pluteus of *Mellita*, but that they possessed tentacles or assisted in capturing food *at this time* is not, I think, supported by evidence. The structure and function of the left hydrocœle as an organ of locomotion and feeding was, in my opinion, acquired during the period of sedentary life after the animal had so increased in size that ciliary action alone was not equal to the work of food gathering. This point will be more fully discussed, however, further on.

To the bilateral ancestor as above described I would add, in the place of the general coat of cilia with which it is usually provided, a locomotor and feeding apparatus consisting of five transversely-placed ciliated rings and an apical tuft of sensory cilia. The position of each is shown in Fig. 11, *a*.

Semon[1] in his discussion of the larva of *Synapta digitata* concludes that the transverse ciliated rings of both the holothurian pupæ and the larva of *Antedon* are to be considered as secondary structures. Lang[2] also states that it is not at all likely that the ciliated rings (of echinoderm larvæ) have any phylogenetic significance. From the facts then available this was the only conclusion warranted and its has been accepted almost without exception by zoölogists. It seems to me however that the observations recorded in this paper, which have been made since the publication of the works of the authors just mentioned, make it worth while to again call attention to the question and to discuss the bearing which the accumulated facts have upon our conception of the structure and history of the hypothetical pelagic ancestor of the echinoderms.

Larvæ such as *Auricularia*, *Bipinnaria*, *Brachiolaria* and *Plutei* have never, to my knowledge, been seriously consided to be primitive although the attempt to establish a relationship between echinoderms and *Balanoglossus*, on account of the general similarity of the movements and external characters of the *Auricularia* and *Tornaria* larvæ, comes very near to an implied belief in their primitiveness. In each of the above-mentioned larvæ we have to do with highly specialized organisms, there being a long period in the life of each during which it is thrown upon its own resources. Its existence during this period depends upon its ability to procure food and escape from its enemies. The rigorous selection which must take place under these conditions can not have failed to have had a profound effect upon the whole organization of the larvæ and especially upon their external characters.

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[1] Richard Semon, "Die Entwicklung der Synapta digitata und die Stammesgeschichte der Echinodernen." *Jena Zeitschrift*, Bd. XXII., 1888.

[2] Arnold Lang, "Text Book of Comparative Anatomy," p. 546. (Eng. trans.)

The whole tendency has been however to look for primitive characters among free-swimming larvæ, throwing aside those which are brooded or otherwise cared for as much more likely to be modified and secondary. If my suggestion as to the significance of the larvæ of echinoderms with transverse rings is correct then this view is incorrect. On the other hand we would expect to find the least modified development among larvæ which are freed, to a greater or less extent, from the task of caring for themselves, provided in such cases the eggs have not been so crowded with nutritive materials as to become greatly enlarged or that, during the brooding, no connections with the mother are established or protective structures developed. Kenogenetic characters are no doubt found in both types of larvæ and the problem is to ascertain which has remained truer to the ancestral form.

The final, sudden and complicated metamorphosis into the adult form which is so characteristic of *free-swimming* larvæ is good evidence that they have been carried far out of the path of phylogeny. In larvæ without a long independent existence the metamorphosis is gradual and such as might be expected if it is in any way a true picture of the past history of the race.

No very great similarity is shown in the external forms of the familiar types of echinoderm larvæ and it is difficult to think of any one of them as having been the type from which the others originated, but it is possible to think of them all as having arisen from a type of larva such as is found in *Antedon*, *Cucumaria* and *Ophiura*.

Owing to the similarity in position of the ciliated rings with reference to the other organs of the body of the larvæ of the above-named species, and all other cases in which ciliated rings have been found, a very definite homology can be shown to exist between them, as I have endeavored to indicate by the numbers which have been placed opposite the rings in each of the drawings. It is conceivable that the long arms of *Auricularia*, *Bipinnaria*, and the various *plutei* may have arisen and been developed from elevations of the ectoderm beneath certain parts of the ciliated rings, the result of which would have been an increase in their length and thereby an increase in their efficiency in locomotion and feeding. The relation which the type of directly developing larvæ of *Antedon*, *Cucumaria* and *Ophiura* is suggested to bear to the familiar types, is shown in Fig. 11; the larvæ with transverse ciliated rings being considered the primitive condition from which the other larvæ have been specialized and carried far out of the path of phylogeny, as a result of their independent life. To this type of development the specialized larvæ tend to return at the time when their free-swimming life is given up for one on the bottom, as is indicated in holothurian pupæ, a certain ophiuran larva and young *Mellitas* in all of which the transverse ciliated rings reappear at the time of metamorphosis in the form in which they were of functional importance to the common ancestor during the early period of its life on the bottom.

All larvæ have not deviated to the same extent from the direct line, as is shown not only in their less complicated structures but also in the less radical readjustment of organs which takes place during their metamorphosis. In ophiurid plutei, for example, the larval mouth and œsphagus are taken over as such into the adult form, which, as has been pointed out, must have been the case in phylogeny. In echinoid plutei, however, the specialization which has taken place in these organs has been carried so far that it is impossible to readapt them to the needs of the adult and new ones must be formed.

THE ATTACHED FORM AND THE ORIGIN OF RADIAL SYMMETRY.

With almost no exception, students of the embryology and anatomy of the echinoderms see no other way, at present, to account for the peculiar asymmetry of certain of the organs and the perfect radial symmetry of other structures, which are characteristic of the adult condition and which arise, in every known case, by the remodelling of the structures of a larva which is bilateral in its entire organization, than by assuming that the group has been derived from a bilateral pelagic organism, similar to the one described above, which at a very remote period in its existence exchanged its free swimming life for one on the bottom during which it became fixed.

Briefly stated, the steps by which the present organization of an echinoderm are generally accounted for, and for which there is more or less evidence, are as follows: Pelagic life was given up for one on the bottom because of less competition and a greater food supply in the latter place. The preoral lobe became gradually modified into an organ for fixation. The mouth, at first directed downward as was its position in pelagic life, gradually moved to the left until it took up a position in which it was directed upward. This, in a fixed animal, feeding upon microscopic organisms, is its most favorable position as is shown by its position in animals which exist at present under these conditions. During the migration of the mouth and œsophagus, those organs of the left side which would obstruct such a movement (left middle and posterior body cavities) were carried along and each became drawn out into the shape of a horseshoe and greatly hypertrophied. In the final position of each, the opening of the horseshoe was directed anteriorly. The middle and posterior body cavities of the right side also became changed in position and correspondingly reduced in size. The left middle body cavity retained its connection with the exterior through the greatly reduced anterior left body cavity and its duct, but the duct of the right side disappeared. During this period when food was plentiful and easily accessible and when no energy was used in locomotion, a rapid increase in the size of the creature took place and radial symmetry was developed. There is such a diversity of opinion, however, as to the details of this process that I will attempt to give but one; that which has been suggested by my own observations.

The ciliated rings were useful to the free-swimming animal not only as organs of locomotion but were used in feeding as well, and during the period when it was fixed on the bottom certain of the rings continued to function as food gatherers. The two rings which encircled the preoral lobe, being purely locomotor in function, were lost, but the other three took up a more definite relation to the mouth and formed six paths for conducting food to it (see Fig. 11, *b*). The retention of the ciliated rings among directly developing larvæ and the return to a condition with ciliated rings among larvæ which possess a more complicated ciliated apparatus during their free-swimming life may, as I have stated elsewhere, be explained on this ground. The entire number of rings is not in every ease retained or reproduced because two, Nos. 1 and 2, belonged entirely to the locomotor apparatus (preoral lobe) and except in the holothurians and crinoids (for reasons suggested later) are no longer needed. Only those rings are retained which in the ancestral line had a function in feeding, and which are needed for the same purpose during the metamorphosis of the larvæ themselves until the developing tube feet are ready to assume the function.

This ciliated feeding apparatus which had been brought over by the hypothetical fixed echinoderm from its free-swimming condition and which, in the new surroundings, had, at first, answered every need in this line, became gradually inadequate to furnish it, as it increased in size, with enough food. Those portions of the ciliated sensory epithelium of the mouth situated between the ends of the ciliated paths were then gradually developed into tentacles into each of which a diverticulum of the left middle body cavity, lying below, protruded (see Fig. 11, *b*). In the anterior space only, did no tentacle develope. This space contained the external opening of the left middle body cavity (madreporite), the left anterior body cavity (*Ampulla*) and possibly the reproductive organ. There was hence no space in which a sixth tentacle might have developed.

In this way the pentamerous structure of the hydrocœle may be accounted for and I assume, with others, that the hydrocœle formed the basis upon which the entire radial symmetry of echinoderms was built. The ciliated tentacles, simple at first, branched as they grew in length and assumed more and more the function of food collecting. As the animal increased in size the space immediately surrounding it failed to yield a sufficient supply of food. The tentacles in reaching about over the bottom in search of more, detached the animal and a crawling habit was developed. As the tentacles grew in length and complexity a like development in the organs which nourish, enervate, support and protect them would naturally follow. The tentacles being five in number, we have in them a possible origin for the pentamerous symmetry which characterizes the nervous and skeletal systems and a considerable part of the cœlomic cavities of all echinoderms. At the time when fixed life was given up by the ancestor of those echinoderms which are at present free living, each of its radii probably contained a five-branched tentacle, since this is the number which is possessed by many echinoderms at the period when their metamorphosis is being completed. The period of fixation was long enough and the changes which took place in the organization of the animal at this time were so great that all trace of an *anterior* or a *posterior* part, as such, was lost and now, in its second period of free life, the direction of locomotion depends wholly upon external conditions.

During the period when the common ancestor of the group was fixed, differentiations into at least three different types took place. One line is now represented by holothurians, one by crinoids and another by asterids, echinoids and ophiuroids. Among crinoids alone the fixed condition has been retained. In this group the problem of enlarging its base of supplies was solved not by becoming free but by the elongation of the organ of attachment, and by the migration of the mouth and tentacles still further toward the opposite end. In the type which has given rise to holothurians, the mouth and tentacles migrated in just the opposite direction, *viz.*, into the organ of attachment and were thereby brought into relation with the bottom. The free-crawling habit was later acquired. The ancestor of the starfishes and sea-urchins made no permanent use of its organ of attachment and no further migration of the mouth took place but it was brought into direct relation to the bottom by the rotation of the body as a whole.

Zoölogical Laboratory of the Johns Hopkins University,
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BIOLOGICAL BULLETIN.

THE SPERMATOGENESIS OF THE MYRIAPODS.—II. ON THE CHROMATIN IN THE SPERMATOCYTES OF SCOLOPENDRA HEROS.

MAULSBY W. BLACKMAN.

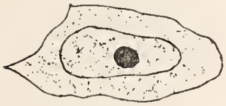
In a detailed study of the spermatocyte changes in *Scolopendra heros*, now practically ready for publication, the multiplicity of subjects requiring consideration is such that it is deemed advisable to prepare a series of shorter papers, in each of which some particular class of structures may be considered to the practical exclusion of the others. It is hoped that in this manner the confusion which necessarily occurs where the whole subject is treated at one time may be avoided. In this, the first of the series of articles, the chromatin structures alone will be treated.

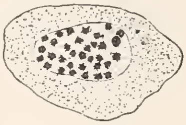
The spermatogonia of *Scolopendra* are small cells of an elongated, irregular shape lying parallel to the long axis of the follicle, and containing an oval nucleus (Fig. 1). During the resting stages the chromatin is all aggregated into one rather large, spherical nucleolus-like body, usually situated at the periphery of the nucleus and apposed to its membrane. The remainder of the nuclear space is filled by an irregular network of granular fibers apparently differing in no way from the cytoplasmic network without the nucleus. In staining reaction the nucleolar body mentioned conforms in all respects to a chromatin body as it indubitably is. When stained with Heidenhain's iron-hæmatoxylin, this structure retains the coloring matter after all other morphological elements of the cell have become almost colorless. In lightly stained preparations evidences appear which warrant the assertion that the body in question is not strictly homogeneous in structure, but probably includes in its composition linin as well as chromatin. With Flemming's three-color method the "nucleolus" takes the dense red stain characteristic of closely aggregated chromatin; and with the Ehrlich-Biondi mixture, following the action of suitable fixatives, assumes the green color usual to chromatin treated by this reagent. Numerous other stains of a greater or less value as micro-chemical tests were used and with all these the chromatin nature of this body was invariably demonstrated.

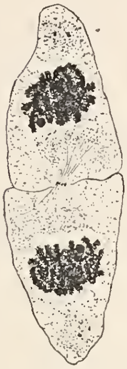
The character of this nucleolar body which, for reasons late made apparent, I shall call the *karyosphere* is still further indicated by its behavior in the prophase of the spermatogonium. Owing to the advanced development of my material I have been unable to study any but the last generations of these cells, but I believe that the phenomena here observed are common to all generations of the secondary spermatogonia. In all cases studied, the active prophase is characterized by the presence within the nuclear vesicle of 33 small aggregations of chromatin and the complete absence of the karyosphere (Fig. 2), thus giving a logical basis to the conclusion that the chromosomes are derived directly from the substances of the karyosphere. Of these 33 chromosomes 32 are characterized in the earlier prophases by their granular consistency, while the remaining one is plainly distinguishable on account of its homogeneous nature and its clear-cut outline. This modified chromatic element is the accessory chromosome, first recognized as a specialized chromosome by McClung, '99, and later found to be probably of universal distribution in the male cells of arthropods.

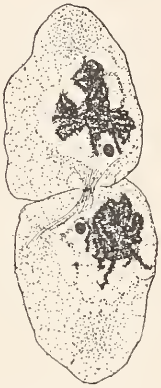
It will be noted that the number of chromosomes, 33, given above as characteristic of the spermatogonium is not a multiple of two as is generally considered to be necessarily the case of immature germ cells. The reason for this fact has to do with the peculiar character of the accessory chromosome, and can readily be explained when the later behavior of this element is known.

During the following phases in the mitosis of the last generation of secondary spermatogonia, nothing of especial interest with regard to the chromatin occurs until the telophase is reached. This phase endures for a considerable time as is shown by the great number of slightly different stages present and by the fact that more spermatogonia are found in this condition than in any other stage of mitosis. In the early telophase where the two new cells are almost completely constricted the chromatin is arranged in a densely packed mass of chromosomes in which the individual elements are indistinguishable. Later (Fig. 3) these elements begin to lose their homogeneous consistency and to lengthen out into densely granular segments. Owing to the dense massing of the chromosomes during this and following stages, the ex-









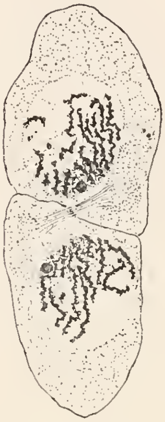


FIG. 1. × 1,440 dia. Spermatogonium of *Scolopendra heros* in the condition of rest. All of the chromatin is aggregated into one mass, the karyosphere.

FIG. 2. × 1,440 dia. Spermatogonium in prophase. The chromatin is all withdrawn from the karyosphere and is now in the form of 33 small chromosomes all of which, with the exception of the accessory chromosome, are of a granular consistency. This element is homogeneous. The centrosomes are to be seen in the cytoplasm near the nucleus.

FIG. 3. × 1,440 dia. Telophase of the last spermatogonium. Synapsis. Cytoplasmic division nearly complete. All chromosomes with exception of accessory, becoming granular. No nuclear membrane. Centrosomes at poles of the cell.

FIG. 4. × 1,440 dia. Later telophase. Synapsis. Chromosomes have lengthened still more. Accessory chromosome still intact. Growth of the cell has begun.

FIG. 5. × 1,440 dia. Early spermatocyte. Nuclear membrane beginning to form. Accessory has taken up a peripheral position. Mass of chromatin has loosened considerably and is now seen to consist of segments equal in number to one half the spermatogonial elements, minus the accessory chromosome. Centrosomes have migrated from their polar position.

act nature of the changes taking place cannot be learned. Several facts are however very apparent. Of these one of the most important is this :—At the time when all the other morphological constituents of the mass of chromatin are undergoing very fundamental changes, one of these elements remains unaltered. While all of the neighboring chromosomes lose their definite outlines and are changed into elongated threads of a granular structure one, the accessory chromosome, does not participate in this metamorphosis but apparently retains all of the properties characteristic of it during metakinesis. While this difference in consistency is the most apparent discrepancy existing between the accessory chromosome and the ordinary chromatic elements, as we shall see presently, it is by no means the most important one.

As the telophase advances the chromosomes continue to lengthen out into long threads. At first, as we have seen, these filaments form a dense mass which is surrounded by no membrane marking off the nuclear area from the cytosome. As the chromosomes become more diffuse this mass also becomes less dense and the individual segments are not so closely apposed to each other. This stage is shown in Fig. 4, where the chromatin of each of the two daughter cells is in the form of an irregular, more or less closely knotted, mass of granular filaments. This mass is contained in a large clear vacuole having no visible network of linin or cytoplasm and bounded by no definite membrane. The appearance of the chromatin grouped in a diffuse mass upon one side of this vesicle suggests very strongly a comparison between this stage in *Scolopendra* and the "synapsis" in elasmobranchs, as described by Moore, '95, and later in different objects by numerous other authors. In all the reported cases with which I am acquainted, however, this massing of the chromatin upon one side of the nuclear vesicle occurs at a considerably later stage than the early or mid-telophase. Paulmier '98, and Montgomery '98, both figure it as taking place after the formation of the chromatic spireme. McClung, '00, denies the normal existence of any such massing of the chromatin in the *Acrididæ*, referring such appearances to the distorting effects of the fixing reagents employed. By the majority of investigators upon male cells this massing of the chromatin is used as the criterion of the synapsis or pseudo-reduction, but Montgomery, '01, apparently abandoning his former views upon the subject, asserts, probably with very good reason, that in reality synapsis occurs at a considerably earlier stage. In *Peripatus*, '00, he is able to study the manner of this union of the chromosomes and from observations seems to have good grounds for the assertion that synapsis is accomplished by an end to end union, in pairs, of entire chromosomes during the retrogressive stages of the telophase of the last spermatogonial division.[1]

In *Scolopendra*, owing to the small size of the spermatogonia and the extreme minuteness of the spermatogonial chromosomes, as well as their larger number and close aggregation during the telophase, the manner of union and the details of the process cannot be studied ; but it can be stated with the greatest certainty that pseudo-reduction occurs during the telophase of the last spermatogonium, and is completed before the reconstruction of the nuclear membrane. At the time of the formation of this structure, the nuclear space is occupied by sixteen elongated segments of chromatin and resembles very closely the nucleus in insect cells with the exception that the nuclear area is much larger in proportion to the amount of chromatin and thus the segmented character of the chromatin is evident (Figs. 5 and 6). Besides these sixteen diffuse segments of chromatin, the accessory chromosome is also plainly visible within the nucleus. It still preserves its distinctive characteristics and has changed very little from its condition during the preceding division. To be sure, it has increased in size as have all parts of the cell, but this increase may all be referred to natural growth. This element takes no part whatever in the process of synapsis. During the spermatogonial stages it is a simple chromatic structure and in the following spermatocyte period it still retains its univalent character when all of the other chromosomes are bivalent.

The completion of cell division and the union of the chromosomes occurring during the telophase have occupied considerable time, as is shown by several facts. Cells in various stages of the telophase are more numerous in the material examined than those in any other condition of the spermatogonium. A large number of different stages may be distinguished. The cells in the early telophase are small, while those in which the nuclear wall is reconstructed are considerably larger, showing that already the growth period has begun. (Compare Figs. 3 and 5.)

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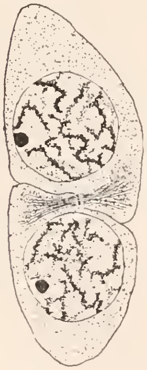
[1] A late paper by W. S. Sutton upon "The Morphology of the Chromosome Group in *Brachystola magna*" contains further and much more convincing proof of the truth of this process. Mr. Sutton is able to trace plainly the union of the chromosomes and to show that it is undoubtedly an end-to-end union of entire elements.

With the completion of the nuclear membrane after the last spermatogonial mitosis, the cells no longer belong to the first division of the spermatogenetic cycle, but now contain the matured number of chromosomes and are spermatocytes. In insect material the transformation is not completed until a period apparently considerably later. However, I believe this difference is merely in appearance, lying in the fact that the nuclear membrane is reconstructed much earlier in insect cells.

At this stage the cells of *Scolopendra* enter upon a period remarkable for the extraordinary changes which take place in their structure. At first glance the most striking of these changes seems to be the enormous increase in the size of the cells (Figs. 6, 7 and 8). This growth I have already described briefly in a preliminary paper and shall have occasion to describe more in detail in subsequent communications. In this connection it will suffice to say that very often the diameter of the larger sperma-



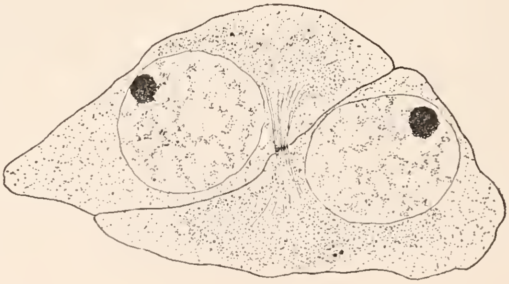


FIG. 6. × 1,440 dia. Slightly later stage. The chromatin segments scattered throughout entire nuclear space.

FIG. 7. × 1,440 dia. Chromatin partly gathered about the accessory chromosome to form the karyosphere. Remaining chromatin of the cell present in the form of very diffuse segments. Spindle remains of last spermatogonial divisions still persist.

tocytes to that of the spermatogonium stands in a ratio of ten to one.

Striking as this great increase in the size of the cells certainly is, it is not as remarkable as are the changes which occur in the cell in general and especially in the nucleus. Shortly after the formation of the nuclear membrane, the chromatin segments leave the tangled mass at one side of the nucleus (Fig. 5), and arrange themselves irregularly throughout the nuclear space (Fig. 6). At the same time they shorten and thicken and, as the nucleus is now quite large, the individual elements may readily be distinguished and their number counted. In all favorable cases in which this count has been taken it has been found that there are seventeen chromosomes present (sixteen granular segments and the accessory chromosome), the number later found in the metaphase. At this time (Fig. 6) the cells resemble insect spermatocytes more closely than at any other stage. They are now in a condition apparently comparable in all particulars to that of the ordinary sperm cell in the "segmented spireme"

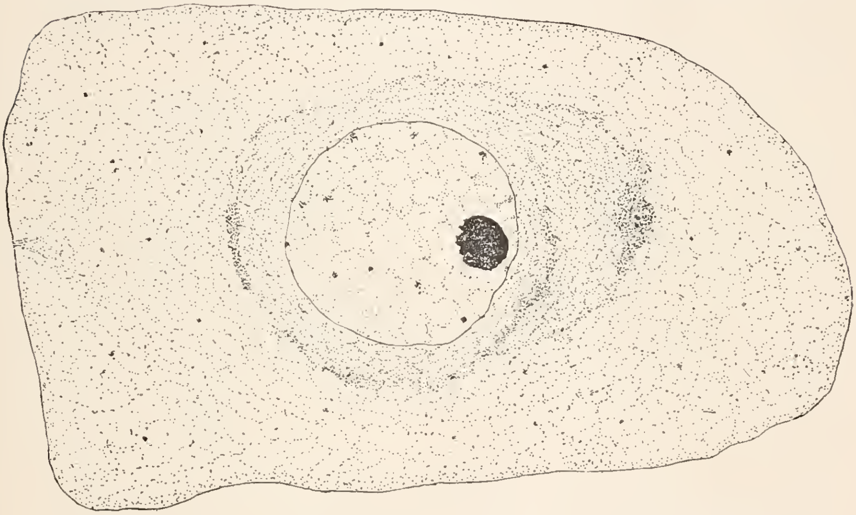


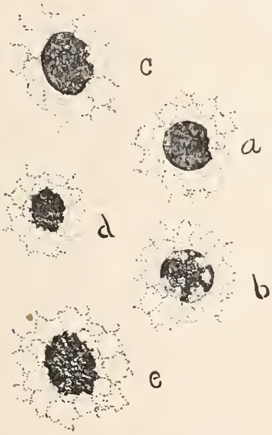
FIG. 8. × 1,440 dia. Pseudo-germinal vesicle stage of the spermatocyte of *Scolopendra heros.* Chromatin all aggregated in karyosphere which here plainly shows except at one point a spongy or reticular structure. This dense portion undoubtedly represents the accessory chromosome. Persisting spindle still visible. Centrosomes to be seen imbedded in the zone of archoplasm surrounding the nucleus.

stage. This is true both with regard to the history of the cell and as regards the morphology of its various structural elements. But from now on the behavior in *Scolopendra* differs very markedly from that of corresponding cells in other animals. In other arthropods at this stage growth is practically completed and the maturation mitoses immediately ensue. In *Scolopendra* the subsequent processes are very different. The growth period has hardly begun and the maturation divisions do not occur until considerably later (probably several weeks or even months). In insects the segmented spireme is considered one of the earlier stages of the active prophase, while in chilopods a condition more closely approaching a true rest stage than that occurring at any other time in the history of the spermatocytes, intervenes between this stage and the first maturation mitosis.

During this intervening stage the history of the spermatocytes parallels in nearly all respects that of the typical female germ cell of a like generation, and the changes which take place result in a structure which if isolated would certainly be mistaken for an immature egg.

As I have reported in a preliminary paper, this resemblance is true not only of the cytoplasmic but of the nuclear elements as well. As the cell continues in its growth the chromatin segments become larger and more diffuse. They no longer retain the stains with the persistency which has characterized them heretofore. This is probably due entirely to the fact that the granules are farther apart and not to a change in the chemical nature of the chromatin. Gradually they break down and their subtance is accumulated about the accessory chromosome, thus seemingly increasing the bulk of this element greatly (Fig. 7). This process continues until finally all of the chromatin of the cell is aggregated in one large intensely staining body situated peripherally in close contact with the nuclear membrane (Fig. 8). The remainder of the nucleus is occupied by a beautiful regular reticulum, the achromatic character of which is shown by the fact that it stains even less densely than the cytoplasmic reticulum immediately without the nucleus.

In a preliminary paper upon *Scolopendra* spermatocytes I stated that I believed this nucleolus-like body to be a homogeneous mass of chromatin. Since then, however, I have studied this structure under more favorable circumstances, and am able to demonstrate that this is not true. In my earlier studies sections six and two thirds micra thick were used and these were studied under a magnification of one thousand diameters. In arriving at my later results thin sections two to three micra thick were used as well as the thicker ones. These were stained in varying intensities with Heidenhain's iron-hæmatoxylin and were studied at a magnification of twelve hundred to eighteen hundred diameters. With these improved conditions it is found that this body, which I shall hereafter call the *karyosphere*, is by no means a simple homogeneous sphere of chromatin, but on the contrary is a rather complex structure consisting of chromatin, linin and karyolymph. It is a mass of fine granular filaments of chromatin so closely gathered about the accessory chromosome as to present, under ordinary conditions and amplification, the appearance of an irregular homogeneous sphere of pure chromatin (Fig. 9, *a*).



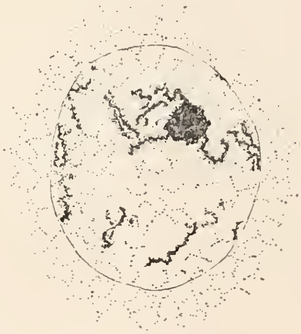


Fig 9. × 1,440 dia. Karyosphere as seen in various preparations; *a*, as it appears in thick densely stained sections; *b*, karyosphere in which the chromatin segments are massed together by the action of the fixing reagents; *c*, thin lightly stained section of karyosphere showing the real normal structure; *d*, section through one side of karyosphere; *e*, karyosphere in early prophase shortly before the appearance of the chromosomes.

Fig. 10. × 1,440 dia. Nucleus of first spermatocyte in prophase, showing the origin of the chromosomes from the karyosphere. A number of segments have already become detached and lie free in the nucleus while others are still connected with the karyosphere. Those detached have already segmented longitudinally.

Upon higher magnification, sections of this karyosphere usually present a granular or spongy appearance as shown in Fig. 9, *c*. In other cases the chromatin is more or less collected into certain areas forming a coarse cluster in the center from which processes extend toward the periphery (Fig. 9, *b*). Here the body still retains its approximately spherical form, the portion between the processes not staining with the chromatin stains but showing the plasma reaction. Quite often, also, we find a karyosphere which presents the appearance shown in Fig. 9, *c*. This I regard as the typical form. It consists of very fine and closely aggregated mass of chromatin filaments arranged in the form of a more or less perfect sphere. Upon one side of this mass when the section is cut through the right plane is a smaller homogeneous body, the accessory chromosome (Fig. 9, *d*, *e*). The remainder of the karyosphere is made up of irregularly arranged chromatic strands between which minute interstices, undoubtedly filled with karyolymph, may be discovered by careful focusing.

Thus it will be seen that during the pseudo-germinal vesicle stage,[1] the karyosphere, with the exception of a membrane, possesses all of the essential elements of a nucleus — chromatin, linin (upon which the chromatin is arranged) and karyolymph. It is in fact a "nucleus within a nucleus" similar to that described by Carnoy in the closely allied genera of chilopods, *Lithobius*, *Scutigera* and *Geophilus*. This structure which he calls the "nucleole noyau," behaves similarly in all essential respects during the first spermatocyte to the karyosphere in *Scolopendra heros*.[2] It is derived from the chromatin of the nucleus in a similar manner and during the first maturation mitosis behaves in a way essentially alike in all respects.

Carnoy by no means stands alone in the assertion that functional chromatin may and does assume the form of nucleolus-like bodies during resting periods between mitoses, although the structures found by him in *Lithobius*, *Scutigera*, etc., are more highly organized than those reported by others. Among those who have observed that the "chromatin nucleolus" is derived from the chromatin reticulum may be mentioned the following: Blochmann, '82 (*Neritina*); Van Beneden, '83 (*Ascaris*); Van Bambeke, '85 (general); Carnoy, '85 (*Arthropoda*); Rabl, '85 (*Salamandra*); O. Schultze, '87 (*Rana* and *Triton*); Davidhoff, '89 (*Distaplia*); Hermann, '89 (*Mus*); McCallum, 91 (*Echinodermata*); Fick, '93 (*Axolotl*); Holl, '93 (*Mus*); Jordan, '93 (*Newt*); Mertens, '94 (*Pica*); Metzner, '94 (*Salamandra*); McCallum, '95 (*Necturus*, also in plants); Sobotta, '95 (*Mus*); R. Hertwig, '96 (poisoned eggs of *Echinodermata*); Carnoy and Lebrun, '97, '98, '99, '00 (*Amphibia*); Eisen, '00 (*Batrachoseps*); Wilson, '01 (chemically fertilized eggs of *Toxopneustes*); and Blackman, '01 (*Scolopendra*). In many of these animals the process has been followed in such detail that no reasonable doubt can exist as to the accuracy of the results obtained. In other cases the conclusions are not so well supported. In several instances all of the chromatin is not withdrawn from the nuclear reticulum. This is especially true of the cells of Amphibia (McCallum, Jordan, Fick, Eisen, *et al.*). In other batrachian cells all of the chromatin is at certain stages collected in a number of granular masses which also contain linin (O. Schultze, Carnoy and Lebrun, *et al.*). In *Mus*, Hermann finds that at first there are several bodies in the spermatid nucleus but these later fuse to form a single large karyosphere. In this he is confirmed by Sobotta.

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[1] See former paper.

[2] Carnoy failed to find a "nucleole noyau" in *S. dalmatica*. He considers the intranuclear body in the cells of this animal a true plasmasome in no way related to the structure found in *Lithobius* and other chilopods.

Other authors state that all of the chromatin of the cell is withdrawn from the nuclear network and deposited in one large "chromatin nucleolus." Such appearances have been observed and carefully studied by Blochmann, Carnoy, Davidhoff, Hermann, Holl, Sobotta, R. Hertwig, Wilson and others. That the results of such well-known investigators should be descredited or received with scepticism seems strange, yet the majority of cytologists seem not to believe that chromatin may normally be massed in a nucleolus-like body and later act as the functional chromatin of the cell.

Now let us inquire whether such scepticism is justifiable? If it can be shown that in the Protozoa such aggregates of chromatin are of common occurrence normally, certainly it is allowable to conclude that at least some metazoan cells should retain this characteristic. With regard to the intranuclear structures of

Protozoa, Calkins has this to say: "A distinct plasmosome or true nucleolus comparable to the analogous structure in Metazoa apparently exists in no case save possibly in *Actinosphærium*, and even here is limited to a passing phase during mitosis (Hertwig, '98). It is probable that the structures which have been almost invariably but erroneously called nucleoli do not belong at all to this category of nuclear elements but represent either the functional chromatin which is aggregated into a central mass (karyosome) during the quiescent or vegetative period of cell life, or the intra-nuclear division center." From the work of Grüber ('83), Rhumbler ('93), Labbe (96), Hertwig (98), Calkins ('98, '01), and others, we must conclude that chromatin bodies resembling nucleoli more or less closely are of very frequent occurrence in unicellular animals. From Calkins' ('01) review of these investigations it is evident that in its primitive condition the chromatin is present in Protozoa in the form of dense homogeneous masses of chromatin (karyosomes) which act as the *nuclei* of these undifferentiated cells. In higher types the nuclei are more complicated. The chromatin may still occur in simple masses, but these are contained within a nuclear membrane which also encloses material other than chromatin (karyoplasm and karyolymph). The spireme condition so characteristic of the chromatin of metazoan germ cells is not commonly found in Protozoa and when present, exists for only a short time.

The karyosomes found in some of the higher types of protozoan nuclei (*Actinosphærium*, Hertwig) are not homogeneous bodies of chromatin, but, besides this substance, also contain linin. This linin often forms a reticulum upon which the chromatin is deposited in the form of granules, an arrangement very similar to that found in the nuclei of metozoan cells, and gives rise to a structure which is similar to the chromatin reticulum of the more differentiated nucleus. It is, however, still more strikingly like the spireme structure of the karyosphere in appearance. That it is different in some respects, however, is shown by comparing the subsequent behavior of the two structures. The differences are what would be expected when we take into consideration the fact that one is contained in a protozoan cell while the other is in a metazoan cell. The chromatin elements are much more firmly established in the higher animals and hence it is to be expected that when the karyosphere breaks down, the resulting fragments should be distinct chromosomes. In protozoa the conditions are different. The chromosomes are not such definite structures and hence when the karyosphere of *Actinosphærium* disintegrates it gives rise to a large number of granules which later collect into chromosome-like masses. However, the relationship is certainly sufficiently close to warrant our placing in the same general category; the solid chromatin nuclei of some Sporozoa and Rhizopoda, the karyosomes of higher protozoan nuclei, and the karyosomes and karyospheres,[1] found in the nuclei of metazoan cells.

"Chromatin nucleoli" being of such universal occurrence in protozoan cells, it is to be expected that some metazoan cells exhibit the same structure. As I have already shown, such examples are fairly common in germ cells and seem to be especially numerous in somatic cells and in the female germinal elements. So far as I know they occur only in cells which are undergoing especially long periods of mitotic inactivity. Such is certainly very evidently true of the germ cells of *Scolopendra heros* where, during the time of their presence, the pseudo-germinal vesicle stage, the cell increases many times in size.

The pseudo-germinal vesicle stage is succeeded by the active prophase of the first maturation division. This phase is inaugurated by modifications in the cytoplasm of the cell and by the migration of the centrosome to the nuclear membrane. Upon reaching this structure the centrosome divides and the two parts begin their divergent courses.

By the time this is well begun the nucleus also commences to show signs of activity. The linin reticulum becomes more ragged and the threads are now composed of finer granules. But the most important phenomena are those to be observed in connection with the karyosphere. At a casual glance this structure seems to have undergone no change, but upon careful examination it is found that its outline is now more irregular and its consistency

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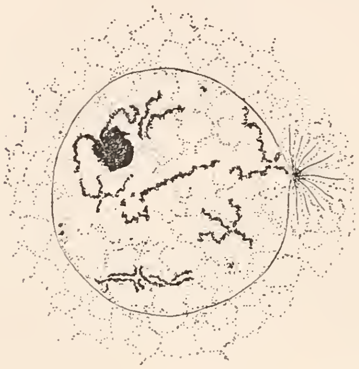
[1] In the above terminology I have limited the term karyosome to structures other than chromosomes found within the nucleus which are apparently composed exclusively of chromatin. The karyosphere is much more highly organized, as it contains chromatin (in a granular, reticular or spireme form), karyoplasm, *i. e.*, linin and karyolymph. It is in fact a miniature nucleus.

more spongy (Fig. 9, *e*). This continues to become more marked until in a short time one or several projections may be seen extending from its surface (Figs. 10, 11). These granular filaments stain densely and are similar in all respects to the chromatin segments characteristic of the "spireme" stage. They continue to lengthen until when they have attained a certain size they become detached from the karyosphere and lie free in the nuclear space (Figs. 10, 11, 12). These segments continue to form until they are exactly equal in number to the threads formerly seen in the early spermatocytes.



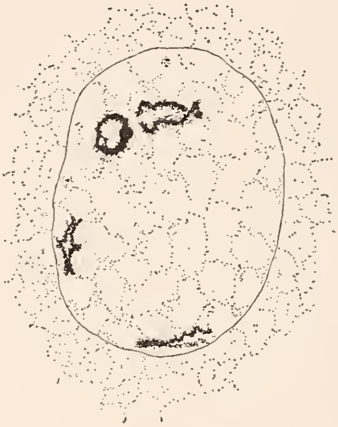


FIG. 11. × 1,440 dia. Nucleus of about the same stage as seen in a thinner section. "Spireme" structure of the karyosphere shown. Tetrads in various stages of formation. One centrosome with rays to be seen upon nuclear membrane, the other not included in the section.

FIG. 12. × 1,440 dia. Later stage, showing the unwinding of the last chromosome from the karyosphere, thus again disclosing the accessory chromosome.

As this process proceeds, the size of the karyosphere decreases proportionately until finally nothing remains except the body with which the transformation started, the accessory chromosome. From this fact alone we might indeed be justified in concluding that the chromosomes are derived from the karyosphere, but no such assumption is necessary. Absolute proof of the truth of this statement is at hand. Actual observations of all the stages incident to chromosome formation may easily be made so that it is impossible for the observer to escape the very evident conclusions to be drawn therefrom. Figs. 10 and 11 are camera lucida drawings of nuclei which show the origin of the chromosomes as well as could be done even by the use of diagrams. In Fig. 10 the karyosphere is very much reduced in size and of an irregular shape; from this three filamentous projections extend, at the distal end of each of which is to be seen a segment evidently only just detached. This is already undergoing the process of tetrad formation. Fig. 12 represents a considerably later stage in which the last chromosome is leaving the karyosphere and the accessory chromosome is again unmistakably to be seen. From these observations I believe no other conclusion can be drawn than that stated above.

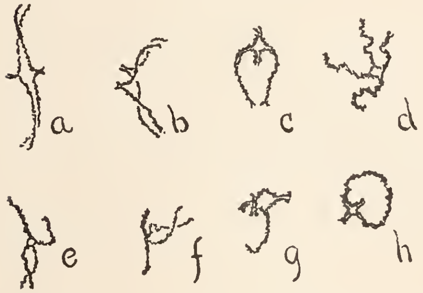
To sum up briefly: At the time of the pseudo-germinal vesicle stage, all the chromatin of the cell is aggregated in the karyosphere which consists of a number of fine chromatin segments closely massed about the accessory chromosome. In the succeeding prophase, the first change has to do with the loosening of this mass of filaments. Later several ends become free and by simply uncoiling, give rise to slender processes extending out into the nucleus. These become detached and new threads are protruded until sixteen segments are present, which together with the accessory chromosome make up seventeen, the number of chromatin elements characteristic of the spermatocytes of *Scolopendra.*

Several investigators mentioned before have traced in considerable detail the origin of chromosomes from nucleolus-like bodies. Blochmann, '82 (*Neritina*) says: "Das die Elemente der Kernblatte aus Theilstücken des Nucleolus entstehen, kann bei unserem Objeke keinen Zweifel unterliegen, da ich alle Uebergangszustände vom unversehrten Nucleolus bis zur angebildeten Kernplatte beobachtet habe." In the germ cells of *Mus* a like condition undoubtedly exists according to the investigations of Hermann, '89; Holl, '93; and Sobotta, '95. Hermann reports "chromatin nucleoli" as present in the cells at various stages of spermatogenesis. Holl shows that in the germinal vesicle of the mouse ovum there is a large nucleolus composed chiefly of chromatin from the substance of which the chromosomes of the first maturation mitosis are formed. Sobotta asserts that during fertilization the chromatin of each pronucleus is in the form of one or several large nucleoli of pure chromatin from which are derived the chromosomes of the succeeding division. In the maturation of the egg of *Distaplia*, Davidhoff, '89, has observed similar phenomena.

C. Schleider, '91, believes that the large nuclei found in the eggs of *Echinodermata* are but reserve masses of chromatin. That this is true under some conditions at least, is shown by the recent experiments of R. Hertwig, '96, and Wilson, '01. Wilson finds that, in one series of eggs chemically fertilized with MgCl solution, the chromosomes functioning in mitosis are obtained by the breaking down of the large densely staining "nucleolus." "Its contour becomes irregular and its texture loose. A little later it assumes a spongy appearance and short irregular processes are extended from its periphery. Enlarging still more it now gives almost the appearance of a close, broken spireme from the ends of which chromatin threads here and there project." These threads later form the chromosomes. As will be readily seen this process in *Toxopneustes* is very similar in many respects to that occuring in *Scolopendra.*

The chromatin segments as they arise from the karyosphere in *Scolopendra* are long, slender, granular filaments usually considerably curved and distorted (Figs. 10, 11, 12). They are arranged irregularly throughout the nuclear area supported by the linin reticulum. Very soon after their detachment from the karyosphere, they are seen to be divided longitudinally along their entire length. Owing to the length and distortion of these segments they frequently assume very fantastic shapes. In some cases the two parts are coiled or twisted about each other like the strands of a rope (Fig. 11) while the two halves of other chromosomes may be separated by a considerable distance (Fig. 10). This cleavage of the segment very evidently represents the longitudinal division of the chromosome, and as the chromosome is first divided in this manner in the prophase it is, I believe, justifiable to conclude with McClung, '00, that the first maturation mitosis accomplishes the equational division of the chromatic elements. Apparently the next change in the structure of the split segments is shown in Fig. 13, *a*. This first becomes apparent as a weakening of the two parts of the segment at about their middle. The threads show a tendency to bend at a more or less acute angle at this point, and this soon results in a transverse division of each of the parts of the chromosome. Thus each of the chromatin segments has been divided into four parts and may from now on be called a tetrad. Following the terminology suggested by McClung, '00, I shall designate each of the parts going to make up the tetrad or chromosome of the first spermatocyte, a chromatid. By this system I believe much confusion will be prevented.

After the cross division has become established the next change observable is shown in Fig. 13 *b*, *c*, *g*. The chromatids revolve upon each other in such a manner that the ends at the point of transverse cleavage are drawn out parallel to each other and an irregular cross-shaped figure is thus formed (Fig. 13, *d*, *e*).



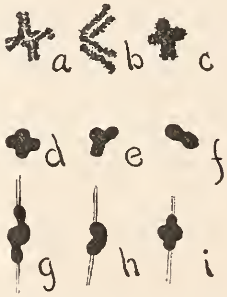


FIG. 13. × 1,400 dia. Various stages and modifications of tetrads. *a*, *b*, *c*, early stages in the process of transverse division. *d*, typical tetrad of mid-prophase. *e*, *f*, *g*, *h*, modifications of the tetrad type.

FIG. 14. × 1,440 dia. Later stages in the history of the tetrad. *a*, typical cruciform tetrad of later prophase. *b*, "double V" form of chromosome at the same stage. *c*, *d*, successively later stages of the cross figure. *e*, *f*, apparent modifications of tetrad in later prophase. *i*, *h*, typical chromosomes at beginning of metaphase. *g*, tetrad undergoing longitudinal division.

This cross-shaped figure is composed of four arms of about equal length each of which is split longitudinally. Owing to the very irregular shape of these arms, the cleavages are masked and are often very hard to demonstrate. However, in later stages when the arms are greatly shortened the bipartite structure is readily seen (Fig. 14, *a*, *b*, *c*), and is also strongly indicated even in the earlier stages by the diamond-shaped opening at the center of the tetrad. When seen *en face* this opening is always square or diamond-shaped with the angles directed toward the arm, indicating that it is continuous into each arm.

At the stage represented in Fig. 13, *d*, the tetrads are often so distorted that the typical form is lost, but upon studying them more carefully it is seen that they are always referable to the same type. Taking *d* as the type, the more common variations are shown in *b*, *c*, *f*, *g*, *h*. At *b* the formation of the arms, instead of occurring in the plane of the threads, has proceeded in a plane at right angles thereto, resulting in the double V figures first mentioned by Paulmier. At *c*, *h* the long arms of the cross have been curved around and nearly brought in contact. Such distortions observed in later stages of tetrads result in a figure similar in shape to a seal ring, the point of double cleavage representing the seal and the long arms approximating to form an apparently closed circle. Fig. 13, *e*, *f*, *g* are but slight or apparent modifications caused by viewing the tetrads diagonally or in profile.

By later changes the arms of the cross figures are much shortened and the divisions between separate chromatids become very apparent (Fig. 14, *a*, *b*, *c*). However, this shortening and condensation continues and these divisions are entirely obliterated and the chromosome becomes first a granular mass and later an apparently homogeneous one. The tetrads even at this stage vary considerably in shape as shown in Fig. 14, *d*, *e*, *f*. The typical form is represented by Fig. 14, *d*, and by numerous chromosomes in Fig. 15.

During the prophase the tetrads of each nucleus have not developed synchronously, but at any given time are in various stages of formation (Fig. 11). This phenomenon is very easily explained. On account of the dense massing of the chromatin segment in the karyosphere, but a few elements can separate at one time and it very naturally follows that those first escaping from this body exhibit more advanced development than those arising later. At a short time before the dissolution of the nuclear membrane, however, the more tardy individuals have overtaken their fellows and all now appear as homogeneous bodies exhibiting strongly all the chromatin reactions.

As will be seen from the foregoing description, the tetrads occurring in *Scolopendra* are similar to those previously described by other authors in various arthropods. What may be taken as the type of these figures was first reported by Paulmier, '99, in Hemiptera and McClung, '00 and '02, in Orthoptera. Structures differing slightly in detail, the apparent divergence evidently being due more to interpretation than to any essential morphological variations, have been found in other arthropods by Henking, '91 (*Pyrrhocoris*), Vom Rath, '95, '97 (*Gryllotalpa*), Toyama, '94 (silkworm), Rückert, '95 (Copepoda), Montgomery,[1] '98, '00, '01 (Hemiptera, *Peripatus*), Blackman, '01 (*Scolopendra*), P. Bouin, '02 (*Lithobius*), Miss Nichols, '02 (*Oniscus*), and others. In the tetrads observed by all of these authors, the cleavages universally represent a longitudinal and a transverse division of the double or bivalent chromosome of the spermatocyte. Apparent discrepancies are undoubtedly due to mere variations in detail or differences in interpretation and denote no real important divergence in the formation of the tetrads.

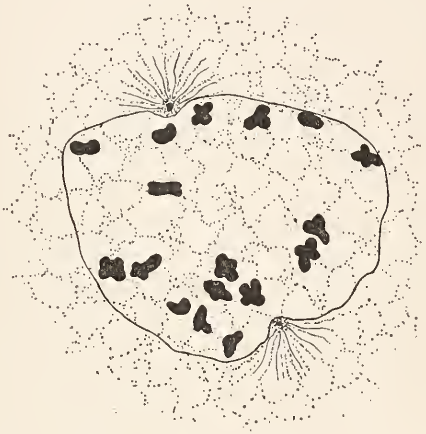


FIG. 15. × 1,440 dia. Nucleus of first spermatocyte during late prophase, showing various modifications in the shape of the chromosomes at this time. The accessory chromosome is seen to be split longitudinally. Centrosomes with well-developed astral rays at opposite poles of the nucleus.

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[1] In his earlier paper Montgomery reported two cross divisions as occurring in *Pentatoma* (*Euchistus*), but in his subsequent publications has denied the accuracy of this observation and now believes that one longitudinal division invariably occurs.

The results of Wilcox, '95, '96 (*Caloptenus*), and de Sinéty, '01 (Orthoptera), however, are indeed radically different. Even here, however, I believe that the divergence is due either to the authors' interpretation of observations, or to insufficient or inferior material. Wilcox asserts that the two spermatocyte mitoses accomplish a double transverse division of the chromosomes. Such is not the case in the western individuals of the same species where a longitudinal, followed by a transverse, division invariably occurs. De Sinéty, working upon the cells of several genera of Orthoptera, asserts that the two divisions are longitudinal. This also appears to be a mistaken conception, as pointed out by McClung, '02. Appearances which upon superficial examination might lead to this view are occasionally met with in Orthopteran material, but when studied closely a different interpretation must always follow. In *Scolopendra* spermatocytes I believe it would be impossible to arrive at this conclusion however strong a preconception the observer may have had. The tetrad figures accompanying this article can by no possibility be logically interpreted as representing anything but a longitudinal and transverse division of the chromosomes. In the interpretation of the first spermatocyte chromosomes and in the sequence of the succeeding divisions I am gratified to note that P. Bouin, working upon other genera of Myriapoda, agrees with my conclusions upon *Scolopendra*.

The tetrad forms which are of most common occurrence in the arthropods are modifications of the cross, double V and ring figures found in *Anasa* (Paulmier, '98) and *Hippiscus* (McClung, '00). It is very probable that all of the other tetrads found in this group are obtained by a greater or less modification of the same process. Such is evidently the case in Copepoda (Ruckert, '94) (Hacker, '95); in *Gryllotalpa* (Vom Rath, '91) and seems also to be true of other invertebrates, *Thalassema* and *Zirphea* (Griffin, '95), *Unio* (Lillie, '95), etc.

The typical arthropod tetrad as exhibited in the Insecta, and in the Myriapoda is obtained in the following manner: The chromatin segments of the matured number as they arise from the spireme stage (Insecta) or from the aggregated segments in the karyosphere (Myriapoda) are long slender threads of granular chromatin. Each thread very quickly splits longitudinally, thus giving rise to two long slender segments extending parallel to each other. Very shortly after this longitudinal split is made, apparent indications of the second cleavage may be seen. The first indication of this is a bending of the two halves of the segment at their middle point. This extension may be in exactly opposite directions when the resulting tetrad is of the typical cross shape or may occur in such a manner that the two angles are drawn out parallel to each other, in which case the double V figure results. This stage of the two forms of tetrad figures is shown in Fig. 13, *a*, *b*. The bending of the two segments soon results in a transverse cleavage at the angles as indicated in Fig. 13, *a*, *b*, *c*, *g*, *h*. The short processes thus produced elongate at the expense of the length of the quadripartite segment until a cruciform figure is produced, the four arms of which are of about equal length. Each of these arms is traversed by a split extending its entire length and thus producing a diamond-shaped opening in the center of the X figure. Thus it is brought about that the two adjacent halves of contiguous arms are continuous and form one of the four chromatids derived by the double splitting of the chromatin segment (Fig. 14, *a*). The structure of the tetrad is best seen in *Scolopendra* in the later stages of tetrad formation when the arms have shortened and when the chromatin granules are more densely grouped together (Fig. 14, *a*, *b*, *c*). In the late prophase the chromosome becomes homogeneous and assumes the four-lobed shape represented in Fig. 14, *d*, *e*, *f*. The diamond-shaped opening at the center and the splits in the arms are entirely obliterated.

While these fundamental changes have been taking place in the other elements the accessory chromosome has also undergone some alteration. As it emerges from the karyosphere this element is a homogeneous spherical mass of chromatin. (Fig. 12). In the late prophase it is no longer spherical but presents the appearance of a rod the two ends of which are constricted (Fig. 15). This constriction undoubtedly indicates a longitudinal division.

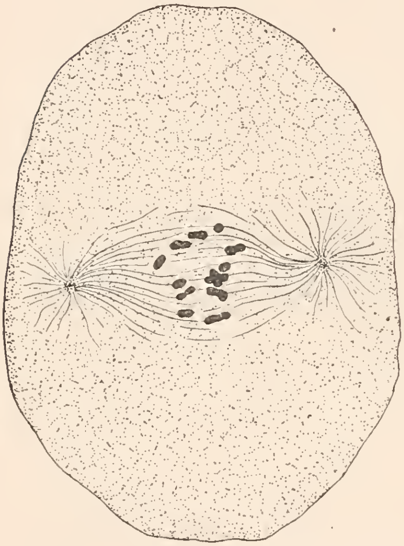
When its history is considered this divergence in form from the tetrads surrounding it is very readily explainable and is precisely what should be expected. Each of the other chromosomes is derived by the fusion of two of the spermatogonial chromosomes during the telophase of the last mitosis of the division period. On the other hand, the accessory chromosome is descended directly from a single element of the spermatogonium. This being true, it is but logical to expect it to behave differently. The primary object of the spermatocyte period is the reduction of the chromosomes to one half the somatic number. It is usually, if not invariably, the case, in arthropods at least, that this period is characterized by two divisions of the chromosomes, a longitudinal and a cross division. It is generally assumed that, by one of these divisions — the transverse division—reduction is accomplished by the pulling apart of the chromosomes at the point at which they were united in the preceding synapsis. Now as the accessory chromosome is not obtained by the union of two spermatogonial chromosomes, this reducing division is not necessary and does not take place. For these reasons while the ordinary chromosomes are each composed of four parts, *i. e.*, are tetrads, this modified chromosome is made up of but two parts, *i. e.*, is a dyad. Furthermore, it is logically to be expected that the accessory chromosome being dyad in its nature would take part in only one of the succeeding divisions. This peculiarity has indeed been observed by many investigators of insect spermatogenesis and several explanations more or less supported by observed facts, are offered in explanation thereof.

In *Scolopendra*, as in other arthropods, the longitudinal division of the chromosomes occurs in the first spermatocyte mitosis. Strong indications of the character of this cleavage may be seen in the metaphase of the first spermatocyte. Fig. 14, *i* represents a typical chromosome at the time of the formation of the first maturation spindle. At *g* is shown a tetrad of the same kind undergoing metakinesis. By a comparison of these two chromosomes it becomes evident that it is a longitudinal division of the element which occurs. The mantle fibers are attached to the two ends, and when the force which separates the halves of the two chromosomes is applied, the two parts glide over each other and seem to separate with the greatest reluctance. The strongest proof that we are here dealing with an equation division, however, is to be found in the prophase. As I have already noted the longitudinal split is the first made manifest at that time, hence logically would be expected to preceed the transverse division, which does not appear until later. Further proof of the sequence of the divisions is found in the second spermatocyte where, as will be presently seen, a cross division of the chromosomes certainly occurs.

The question as to the sequence of the two spermatocyte division, while probably of not any vital importance, has been the subject of considerable controversy. By far the greater number, however, agree that the equation division comes first, and is succeeded by the reduction division. Ruckert, '92, Hacker, '92, McClung, '00, '02, Blackman, '01, P. Bouin, '02, in arthropods, and Bolles Lee, '97, Linville, '00, Griffin, '99, Klinckowström, '97, Francotte, '97, and Van der Stricht, '98, in other invertebrates, have arrived at this conclusion. While the opposing view — *i. e.*, that the reduction division precedes — is held by Vom Rath, '92, '95, Henking, '90, Paulmier, '99, and Montgomery, '98, '00, '01, in anthropods and Lillie, '01, in molluscs. In arriving at this latter conclusion the criterion invariably used is the appearance and behavior of the elements during the two mitoses. But during the metaphase the chromosomes are always so compact that the cleavages shown in the prophase are entirely obliterated, and the manner of division therefore cannot be determined with certainty. An example of the likelihood of misinterpretation of the nature of these divisions is shown by Griffin, '99, *Thalassema*. Here the first division is very evidently longitudinal, and upon superficial observation the second also appears to be of the same nature. But when the phenomena observed in the prophase are considered, it is evident that this cannot be true, as an indubitable transverse cleavage was to be seen at that time. Upon further study Griffin shows his first impression to be false, for the second division is in reality a reducing division.

In all of the investigations with which I am acquainted it has been reported that the longitudinal cleavage is the first to be made evident in the prophase. Then I believe it is but logical to conclude that this division is completed by the first spermatocyte mitosis, especially when this has been shown to be the case in a great number of cells. Of course it is possible that the process varies in different animals, but it is not probable, for if the sequence of the actual divisions varies, we should naturally expect the prophase phenomena to vary in a like manner. No such variation seems to exist.

The chromosomes as they occur in the metaphase are arranged in no definite equatorial plate but are scattered irregularly throughout the equatorial region of the spindle (Fig. 16). It is also noticeable that the chromosomes do not divide synchronously. While some still plainly show their tetrad character, others have completed their separation and have already started toward the poles.



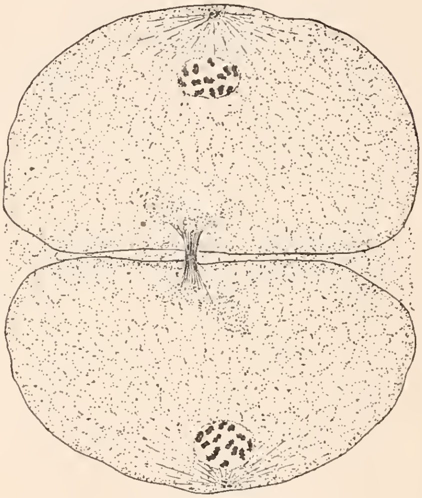
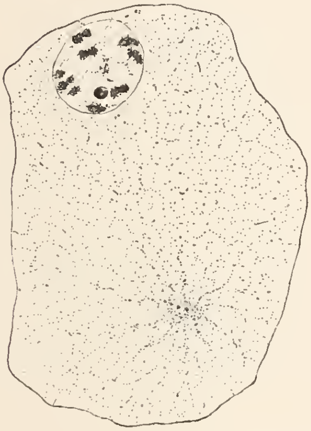


FIG. 16. × 960 dia. Early metaphase of first spermatocyte. Showing the diversity in shape of the chromosomes, and their irregular arrangement in the equatorial region.

FIG. 17. × 960 dia. Telophase of first spermatocyte, showing the unequal division of the chromatin, the accessory chromosome being present in one cell while it is absent in the other.

Owing to the approximately equal size of all the chromosomes and the diversity of shapes which they present it has not been found possible to trace the history of the accessory chromosome during the first metakinesis. However, from an examination of the telophase succeeding, it becomes evident that this element in *Scolopendra* undergoes processes analogous to those reported in insects by a number of investigators. It is found in one of the cells resulting from the first mitosis and does not occur in the other (Fig. 17) showing that it takes no part in this division but goes over to one cell undivided.

With the reconstruction of the daughter nuclei, all of the chromosomes except the accessory become granular (Fig. 18) and present the appearance of rather short rods of diffuse chromatin, the center of each of which is slightly constricted, thus producing a dumb-bell-shaped body. In the succeeding stages these become more dense (Fig. 19) and finally go to the equatorial plate as small homogeneous bodies of a distinctly lobate structure. When arranged in the equatorial region (as in the first division, there is no true equatorial plate) the lobes of these bodies are directed toward the poles of the spindle, thus giving further basis for the conclusion that we have here a cross division of the chromosome.



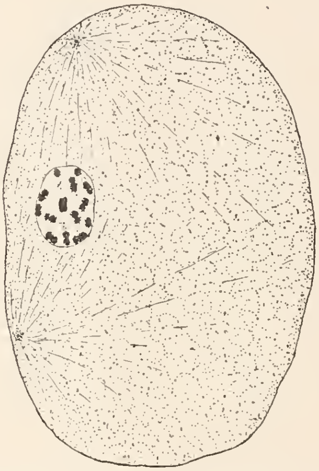
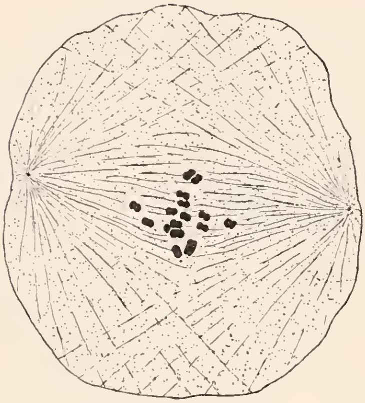
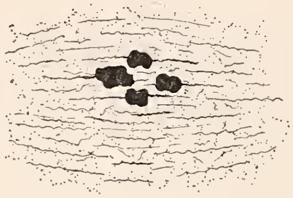


FIG. 18. × 960 dia. Prophase of a second spermatocyte containing the accessory chromosome. The ordinary chromosomes are diffuse and of a dumb-bell form, while the accessory is homogeneous and spherical. Centrosome and persisting archoplasm visible.

FIG. 19. × 960 dia. Late prophase of second spermatocyte. Chromosomes are less diffuse. Accessory chromosome seen to be constricted longitudinally, while the others show indications of a transverse division.

During the metaphase, however, one of the chromatic elements does not show the dumb-bell-shape characteristic of the rest, but is very evidently a rod split in the opposite direction, *i. e.*, longitudinally. This peculiarity is also to be seen in the preceding prophase where the accessory chromosome is of the same shape as in the first spermatocyte prophase. As it is seen during the early metaphase, this element is arranged with the plane of cleavage at right angles to the spindle (Fig. 20), but upon the contraction of the mantle fibers which are attached to opposite ends of the element it revolves through an arc of 90° (Figs. 21, 22) and the component chromatids as they are pulled apart seem to glide over each other (Fig. 22) in a manner similar to that already noted as characteristic of the ordinary chromosomes during the first mitosis.





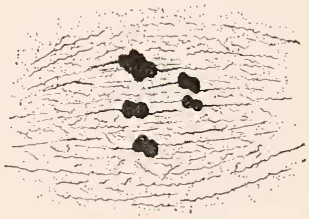


Fig. 20. × 960 dia. Metaphase of second spermatocyte. The difference in shape and orientation existing between the accessory and the other chromosomes is evident.

Fig. 21. × 1,920 dia. High magnification of same stage, showing the differences exhibited by the accessory chromosome in the relation of the chromatids and in the attachment of the mantle fibers.

Fig. 22. × 1,920 dia. Slightly later stage; showing the effect of the contraction of the mantle fibers on the orientation of the accessory chromosome.

It will be seen by consulting the accompanying figures that the behavior of the other elements is quite different. These are arranged with their long axis parallel to that of the spindle, the separation of the chromatid occurring along the equatorial plane at the place of constriction. This very evidently represents a cross division of the chromosome.

In the division figures of one half of the second spermatocytes, all the chromosomes are of one type (the dumb-bell form), the accessory chromosome not being present. Thus it will be readily seen that the cells arising from two spermatocyte mitoses are divided into two classes of equal numbers — those which possess the accessory and those which do not. Similar phenomena have been observed in the cells of a number of insects by Henking, '90, Paulmier, '99, McClung, '00, '02, de Sinéty, '01, and others.

Regarding the function of the modified chromosome, two theories have now been advanced. Paulmier in his paper on *Anasa* puts forth the theory that the "small chromosome represents characteristics which are being eliminated from the race." He bases this conclusion entirely upon the failure of the element to divide in one spermatocyte division. Montgomery in his later papers adopts the conclusions of Paulmier and believes with him that it is a chromosome undergoing the process of elimination.

McClung, '02, however, in a paper in which he considers in detail all of the reported observations upon the accessory chromosome, formulates an hypothesis which ascribes a very different function to this element. He maintains that the mere fact of the unequal apportionment to the spermatozoa would not necessarily indicate that the element is degenerating, and in addition there are other facts which militate strongly against such a conclusion. The extreme nicety with which this element is excluded from contact with the others in most stages, especially in the spermatogium, would seem to indicate a very different and much greater significance. This exclusiveness, taken in connection with the fact that exactly one half the spermatozoa contain the accessory chromosome, suggests the theory that it has to do with the determination of sex, as this is the only respect in which the progeny are divided into two classes of equal numbers. Although no positive proof is advanced to support this theory the author establishes in a very logical manner the probability of the accessory chromosome representing such a function. It seems to possess all the characteristics required of such an element. My observations upon *Scolopendra* surely lend support to this theory. Definite proof of the function of this structure can only be obtained, however, by a study of the process occurring in the fertilization and cleavage of the egg.

My observations upon the accessory chromosome in *Scolopendra* have added very little to our knowledge of this element, except in so far as they help to show its wide distribution and the great similarity of its behavior in widely separated groups. Indeed in all important particulars the phenomena accompanying the development of this structure are identical in Chilopoda and Orthoptera, although the minor details of the process vary considerably. In both groups the element is derived directly from a single spermatogonial chromosome, and for this reason takes no active part in the phenomena of synapsis. During the prophase when the other chromosomes divide into four chromatids and form tetrads, this element, as would be expected from its origin, cleaves but once and that longitudinally. In the two succeeding divisions it is divided but once and thus is present in but one half of the spermatids. The differences, although at times puzzling, are in reality slight and unimportant. Thus, at the time when all of the chromatin is aggregated in the karyosphere, the accessory chromosome cannot be distinguished except in the most favorable cases ; but from the study of these thin, well-differentiated sections we are justified in saying that even in the pseudo-germinal vesicle stage this element retains all its ordinary characteristics. In the mataphase of the first spermatocyte it cannot be distinguished from the other chromosomes as it can in Orthopteran material, because it is of approximately the same size as these. In the second maturation division, however, it is again very evident, by reason of the fact that it divides longitudinally while the other chromosomes divide transversely.

These variations, as has been said, are unimportant modifications of behavior and do not represent such fundamental differences as seem to exist between the "small chromosome" (Paulmier) or the "chromatin nucleolus" (Montgomery) in Hemiptera and the accessory chromosome in Orthoptera. If the observations of Paulmier and Montgomery concerning the origin of this element are correct, it is indeed doubtful whether the bodies described represent the same structure as the accessory chromosome. The chromosome *x* of *Protenor* (Montgomery, '01) would seem more closely to approach this modified element in origin and behavior.

I am glad of this opportunity of expressing my gratitude to Dr. C. E. McClung for valuable advise and criticism throughout the progress of this work.

Laboratory of Zoölogy and Histology,
University of Kansas, April 11, 1903.

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THE EFFECTS OF HEAT ON THE DEVELOPMENT OF THE TOAD'S EGG.

HELEN DEAN KING.

An extended series of experiments made by Hertwig (1–4) prove that the maximum temperature at which the eggs of the frog will develop normally differs for different species. His experiments also show that eggs in the cleavage stages can withstand a higher temperature than can unsegmented eggs. These results have a bearing on the general problem of adaptation; for it may be possible to show, after more species have been studied, that the maximum temperature which the eggs of amphibians can endure without injury and also the temperature most favorable for their development depend, to a certain extent at least, on the time of year at which the eggs are deposited.

Material and Method.

The eggs of the common toad, *Bufo lentiginosus*, were used in making all of the experiments recorded in the present paper. After natural fertilization, the eggs were brought into the laboratory where the temperature varied from 18 to 21° C. Control sets of eggs from each lot used for the experiments, developing at the room temperature, all became perfectly normal embryos, and some of them were kept until metamorphosis.

In making the experiments, small dishes containing about 80 c.c. of spring water were placed in the drying chamber of a large water-bath, and after the water had become heated, from 50 to 75 eggs were quickly transferred into it and left a given length of time. The temperature to which the eggs were being subjected could readily be told from a thermometer that projected into the chamber through a small opening in the top. Great care was taken to keep the temperature of the chamber as constant as possible during the course of the experiments, and in no case did it vary more than two degrees. After the eggs were removed from the chamber, they were put into fresh water at room temperature and their later development compared with that of the eggs in the control set.

II. Experiments on Unsegmented Eggs.

Experiment 1. — On April 16, twenty-five unsegmented eggs were subjected to a temperature of 28–30° C. for two and one-half hours. When removed from the chamber, all of the eggs were in the 16-cell stage, while in the control set, developing at room temperature, the eggs had only reached the 4–8-cell stage. The immediate effect of the higher temperature, therefore, was to increase the rate of development. This result agrees fully with that obtained by Hertwig in many of his temperature experiments on the frog's egg. The later development of the eggs in this series appeared to be perfectly normal, and it took place at about the same rate as in the eggs of the control set.

Experiment 2. — A number of eggs that had not yet segmented were put into water at a temperature of 30–32° C. on April 17. Part of the eggs were removed at the expiration of three quarters of an hour, and when examined they were all found to be segmenting. In a few cases the first cleavage plane had nearly cut through the yolk portion of the egg and the second furrow was appearing. In the control set of eggs, the first cleavage plane was just coming in at this time, so that, in this experiment also, the early development became more rapid as an immediate result of exposing the eggs to a higher temperature. All of these eggs developed into normal embryos.

Some of the eggs of the above lot remained in the heated chamber for one hour. The second cleavage plane had appeared in all of the eggs when they were removed to room temperature. Later segmentation was normal, and on the following day the dorsal lip of the blastopore appeared in all of the eggs at about the same time that it formed in the eggs of the control set. On April 19, many of the eggs were dead; some were in the early gastrula stages, and some showed traces of the medullary folds. Of the seven embryos alive on April 20, three were abnormal, having a large yolk plug exposed at the posterior end of the body; the other four embryos were normal and were kept for several weeks.

The remaining eggs of this lot were kept at the temperature of 30–32° C. for one and one-half hours. At the end of this time they were in the 16-cell stage, while the eggs of the control set were only in the 2–4-cell stage. Later segmentation of these eggs seemed to be normal, and on April 18 the dorsal lip of the blastopore appeared in a very few of them. On the morning of April 19 most of the eggs were dead, and not one of them, when examined, was found to have gastrulated. In the eggs still living the blastopore was closing in, but development was much slower than that of the eggs of the control set in which, at this time, the blastopore had already closed and the medullary folds were forming. All of the eggs were dead on the morning of April 20, and in no case was gastrulation entirely completed.

In these last two lots of eggs the injurious effects of heat were not apparent during the segmentation stages and only manifested themselves when the eggs were ready to gastrulate. Early development was accelerated; but later development lagged behind, or, at most, was equal to that of the eggs in the control set.

Experiment 3. — A number of unsegmented eggs were exposed to a temperature of 32° C. for two hours on April 22, and when removed they were in the 16-cell stage. In this lot of eggs the later cleavage was very abnormal as the upper hemisphere divided into a number of small cells, while the lower part of the egg segmented only a few times and, consequently, was composed of a small number of very large cells. Cleavage lines were very distinct in the upper part of the egg; but it was almost impossible to make out the boundaries of the yolk cells. None of the eggs in this set gastrulated and all of them were dead by April 24.

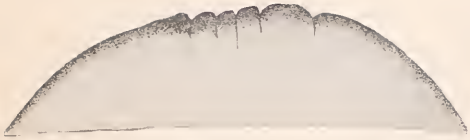
Experiment 4. — On the morning of April 16, a small lot of eggs was subjected to a temperature of 32–33° C. for one-half of an hour. The eggs had not segmented when they were put into cooler water, but in every case the first furrow appeared in about fifteen minutes. In the control set, the first cleavage plane came in about half an hour later than it did in the eggs used for the experiment. All of the eggs of this set developed normally, and sections made of later embryos showed them to be no different from the embryos of the control set.

Experiment 5.— A bunch of about seventy-five unsegmented eggs was put into water heated to a temperature of 34–35° C. on April 16. Part of the eggs were removed at the end of half an hour and a few of them at once began to segment. None of the cleavage planes, with the exception of the first, came in normally, and in no case did any of them cut through the entire egg. Part of a section of one of these eggs is shown in Fig. 1. All of the cleavage planes are seen to be parallel and to extend but a short distance through the upper hemisphere of the egg. Development did not progress beyond this stage in any case, and the majority of the eggs never segmented although they appeared to be living several hours after they were brought into room temperature.

Some of the eggs of the above lot remained at the temperature of 34–35° C. for one hour. When put into cooler water and examined, a slight depression was found in the center of the upper hemisphere of a few of the eggs as if the first cleavage plane was about to appear in its normal position. This appearance, however, proved to be only a wrinkling of the surface as none of the eggs, when sectioned, showed any true cleavage planes.

The above experiments show that the unsegmented eggs of the toad can withstand a temperature of 32–33° C. for one-half of an hour and develop normally, while an exposure to this temperature for a longer period is very injurious and only a small per cent. of the eggs produced normal tadpoles. Exposure to a temperature of 34°, even for a short time, injures the eggs beyond the possibility of a recovery. The maximum temperature that the unsegmented egg can endure without injury is, therefore, 33° C. The optimum temperature, a term defined by Hertwig (3) as, "Die Temperatur bei welcher sich der Entwicklungsprocess bei allen Eiren mit der grössten Beschleunigung ohne eine auffällige Störung und Abweichung von der Norm vollzieht," for this egg is probably not far from 28° C., judging from the results obtained in experiments 1 and 2. In all cases in which the heat did not kill the eggs, development was accelerated at first, apparently with no injurious effects on the egg. In later stages,

1



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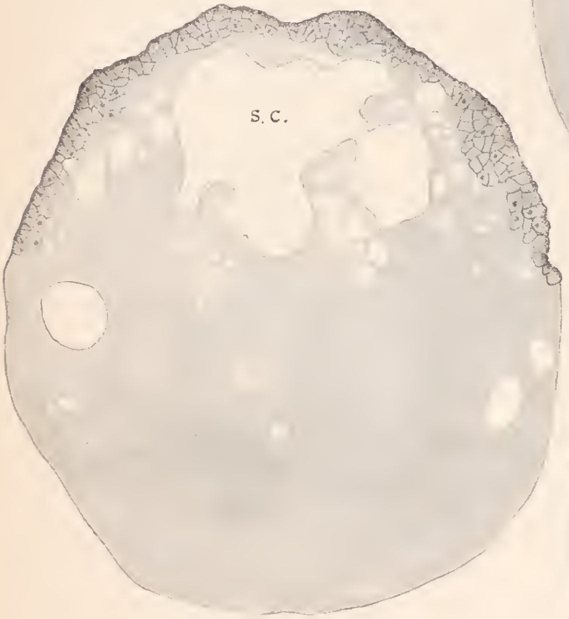

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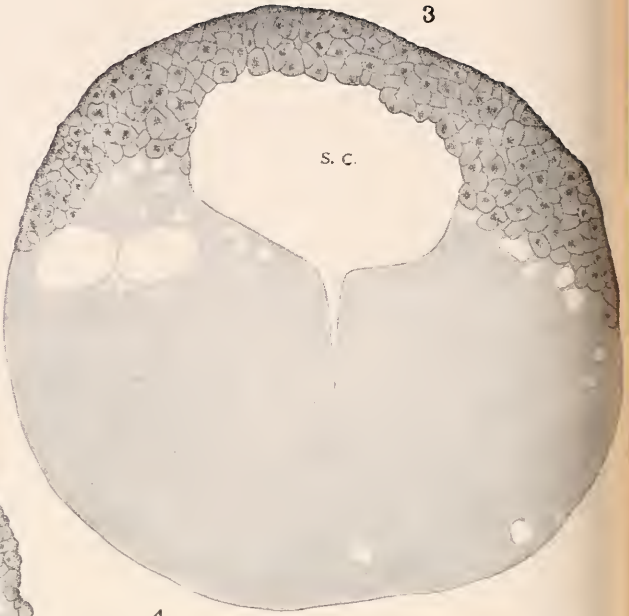

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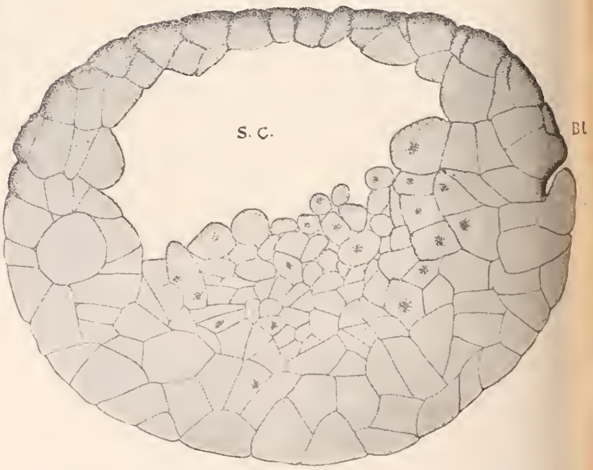

FIG. 1. Part of a section of an egg that was subjected to a temperature of 34–35°C. for one-half of an hour before cleavage began.

FIG. 2. A section of an egg that was exposed to a temperature of 35–36° C. for three quarters of an hour when it was in the two-cell stage. *S. C.*, segmentation cavity.

FIG. 3. A section of an egg that was subjected to a temperature of 33–35° C. for two hours after the first cleavage plane had appeared.

FIG. 4. A section of an egg that was subjected to a temperature of 31–33° C. for three and one-half hours when it was in the 32–64-cell stage of development. *Bl.* blastopore.

however, the eggs of the control sets appeared to be fully as far advanced in development as were the eggs that had been subjected to a higher temperature. Increase in the rate of development is, therefore, but the immediate effect of heat, and after the eggs are brought into a lower temperature they develop at the same, or a lower rate, than the eggs of the control set.

III. Experiments on Eggs in Early Cleavage Stages.

Experiment 6. — On April 17, a lot of about fifty eggs in the 2-cell stage of development was exposed to a temperature of 31–33° C. At the end of one and one-half hours, part of the eggs were removed. They were then in the 8–16-cell stage. The later development of these eggs was perfectly normal in every respect.

The rest of the eggs of this lot remained in the heated chamber for two hours. All of these eggs developed normally during the early cleavage and gastrulation stages; but later a few embryos were found with shortened medullary folds and a large yolk plug at the posterior end of the body. This form of abnormality is very common among embryos that have been injured by exposure to heat.

Experiment 7. — As the first cleavage plane was appearing, a lot of about fifty eggs was subjected to a temperature of 35–36° C. for three quarters of an hour. All of the eggs were segmenting in a very abnormal manner when they were transferred into water at the room temperature, and none of them ever gastrulated. Fig. 2 shows a median section through one of these eggs. With the exception of the layer of small cells bordering the outer surface of the upper hemisphere, the entire substance of the egg is seen to be unsegmented and to have a number of different sized vacuoles scattered through it. A large, irregularly shaped cavity fills the greater part of the upper hemisphere of the egg. This cavity is much larger than the segmentation cavity in a normally segmenting egg, and it appears to be formed of the true segmentation cavity and several large vacuoles which have come to open into it.

Experiment 8. — On April 22, a lot of eggs in the 2-cell stage was exposed to a temperature of 35–36° C. for one hour. When removed from the chamber the eggs were in the 8-cell stage, but development stopped at this point and all of the eggs were dead inside of twenty-four hours.

Experiment 9. — In this experiment, eggs in the 2- and in the 4-cell stages of development, were subjected to a temperature of 33–35° C. for a period of two hours. At the end of this time the eggs were segmenting very irregularly in the upper hemisphere and no cleavage planes were visible in the yolk portion of the egg. A section through one of these eggs (Fig. 3) shows the entire upper hemisphere divided into a mass of small cells containing a considerable amount of pigment which is, for the most part, collected in the middle of the cell around the nucleus. The first cleavage plane has cut only partially through the yolk portion of the egg, as its progress was evidently stopped at the beginning of the experiment. There are no nuclei in the yolk portion of the egg, and the many vacuoles show the injurious effects of the heat. The mass of small cells in the upper hemisphere forms a sort of cap on the unsegmented yolk and make it appear as if the segmentation of the egg was meroblastic. This same sort of abnormal cleavage has also been obtained by Hertwig (1, 2).

According to the experiments in this series, eggs in the early cleavage stages can endure exposure to a temperature of 31–33° C. for a longer period than can the unsegmented egg; yet they are permanently injured by even a short immersion in water at a temperature of 35°. The maximum temperature for these eggs, therefore, is not greater than that for the unsegmented egg. Hertwig (4) has found that the maximum temperature for the eggs of *Rana fusca* in the 8-cell stage of development is 26–28°, which is 3–4° higher than that for the unsegmented egg.

IV. Experiments on Eggs in Late Segmentation and Early Gastrula Stages.

Experiment 10. — On April 18, fifty eggs in the 32–64-cell stage of development were kept at a temperature of 31–33° C. for two hours. Subsequently all of the eggs developed into normal embryos and at about the same rate as did the eggs of the control set.

Experiment 11. — Another set of fifty eggs from the same bunch as the eggs used in experiment 10, was subjected to a temperature of 31–33° C. for three hours. The late segmentation and early gastrulation stages of all of these eggs seemed to be perfectly normal. Two days after the experiment was made, 38 of the eggs were dead, the blastopore not having closed in any case. Of the remaining eggs four only were normal, the rest had a large yolk plug at the posterior end of the body.

Experiment 12. — Twenty-five eggs from the same lot as those used in the two preceding experiments remained in water at a temperature of 31–33° C. for three and one-half hours. Fifteen of the eggs died in the blastula stage. The blastopore appeared in the other ten eggs, but in many cases it was in an unusual position at the equator of the egg. When the dorsal lip of the blastopore was forming in these eggs, the circular blastopore was already beginning to close in the control set of eggs, therefore, in this instance, the heat retarded instead of increased the rate of development of the eggs. In none of the eggs of this set did the blastopore ever become circular, and all of the eggs were dead two days after the experiment was made.

Fig. 4 shows a section of one of these eggs preserved when the blastopore appeared in surface view as a short, straight line at the equatorial zone. The dorsal lip of the blastopore rarely, if ever, comes in as high up as the equator in eggs that are developing normally ; but it sometimes occupies an unusual position in eggs that have been subjected to abnormal conditions. Morgan (5) has found the blastoporic rim above the equatorial zone in eggs of *Rana palustris* that have been subjected to intense cold. In Fig. 4 the archenteron appears as a shallow depression with its dorsal wall formed of heavily pigmented cells as is normally the case. The inner end of the archenteron, instead of turning up towards the black pole as it would do in a normal egg, here projects downward towards the yolk pole. The most interesting fact shown by the section is that the normal position of the large and of the small cells of the egg is completely reversed. In normally gastrulating eggs, the roof of the segmentation cavity is formed of two to three layers of small, pigmented cells, while the ventral wall is composed entirely of large yolk cells that contain little, if any, pigment. In this egg, however, the upper wall of the segmentation cavity is made up of a single layer of heavily pigmented cells which are fully as large as any other cells in the egg. Below the segmentation cavity, a portion of the yolk is divided into a number of small cells, many of which contain pigment massed around the nucleus. Some of these cells are rounded and seem to lie free in the segmentation cavity, an appearance also noted by Hertwig (4) in eggs of *Rana fusca* that were exposed to a temperature of 29–35° C. after having reached about the 100-cell-stage of development.

Morgan has also noted the relatively large size of the cells in the upper hemisphere of gastrulating eggs of *Rana palustris* that had been subjected to cold. He suggests that this increase in the size of the cells "may be due in part to the absorption of water by the individual cells," and he adds that, "even if this is the case the cells are fewer in number than in a normal egg beginning to gastrulate." In the figure shown by Morgan, the cells of the lower hemisphere are all considerably larger than those of the upper hemisphere; the egg, therefore, must have been much more normal than the one from which Fig. 4 was drawn.

It is evident, in the case of the egg shown in Fig. 4, that the increased temperature did not injure the yolk region or retard its development as is usually the case in these experiments; on the contrary, it is the segmentation of the upper hemisphere that has been delayed, while the segmentation of the lower portion of the egg has continued. No egg in this set of experiments developed much beyond the stage represented by Fig. 4, and each of the ten eggs that were sectioned showed abnormalities of the same general character.

Experiment 13. — On April 26, about seventy-five eggs in the late blastula stage were subjected to a temperature of 33–35° C. A part of the eggs were removed at the end of one and one-half hours and they all developed into normal embryos.

A second portion of the eggs was exposed to this temperature for two and one-half hours. All of these eggs developed into normal embryos, although somewhat more slowly than did those of the control set.

A third part of the eggs remained at the temperature of 33–35° for three and one-half hours. These eggs were all dead when removed from the influence of the heat.

Experiment 14. — A number of eggs in the blastula stage were exposed to a temperature of 36–37° C. on April 26. Some of the eggs were removed from the chamber at the end of one-half of an hour. The eggs did not appear to be injured in any way by the experiment and all developed normally.

A second portion of the eggs from the above lot remained at this temperature of 36–37° C. for three quarters of an hour. All of the eggs gastrulated normally, but about half of them died before the blastopore closed. When sectioned these eggs showed no abnormalities. The rest of the eggs became normal embryos, although developing very slowly. The medullary folds had closed in the eggs of the control set when they were only beginning to unite in the eggs that had been subjected to the increased temperature.

The remaining eggs of this lot were removed to room temperature at the end of one hour. Although the eggs did not appear to be dead when they were examined, they did not gastrulate and none of them were alive the day following the experiment.

Experiment 15. — Twenty eggs in late segmentation stages were subjected to a temperature of 40–42° C. for one quarter of an hour. Development was at once stopped by the heat, and all of the eggs were killed.

Experiment 16. — When the dorsal lip of the blastopore was just appearing, a lot of about twenty eggs was put into water at a temperature of 33–35° C. and left there for three hours. All of the eggs continued to develop somewhat more slowly than the eggs of the control set and all became normal embryos.

Experiment 17. — On April 24, a lot of eggs in early gastrulation stages was kept at a temperature of 35–37° C. for one hour. In all of the eggs the lateral and ventral lips of the blastopore formed in the normal manner, but development stopped at this point and the eggs died. No abnormalities were detected when sections were made of several of these eggs.

Experiment 18. — Eggs in early gastrulation stages were exposed to a temperature of 37–38° C. on April 24. A part of the eggs were removed at the end of one quarter of an hour. None of these eggs seemed to be injured in any way by the high degree of heat to which they had been subjected and all developed, somewhat slowly, into normal embryos. The rest of the eggs in this lot remained at the temperature of 37–38° C. for one hour. They were all dead when removed to room temperature.

The results of the experiments in this series show that eggs in the 32–64-cell stage cannot withstand a temperature of 31–33° C. for a much longer period than can eggs that have just begun to segment. The maximum temperature to which eggs can be subjected without injury is practically the same for unsegmented eggs and for those in early cleavage stages, although eggs in the later stages can remain at this temperature for a somewhat longer period and still develop normally.

Eggs in late cleavage stages have a much greater power to withstand high temperature than have eggs in the earlier stages of development, as they will develop normally after exposure to a temperature of 36–37° C. for one-half of an hour. The maximum degree of heat that can be endured without injury is still higher for eggs in the gastrula stages, as they become normal embryos after being subjected to a temperature of 37–38° C. for one quarter of an hour.

The experiments described above are summarized in the following table. The number of the experiment is given in the first column; the condition of the eggs when the experiment was begun in the second column; the temperature to which the eggs were subjected in the third column; followed in the next two columns by the duration of the experiment and a brief statement of the results.

The results of these experiments are very similar to those obtained by Hertwig (1–4) in his study of the effects of heat on the development of the eggs of various species of frogs; and the abnormalities produced resemble, in many respects, those which Hertwig has described and figured. When the unsegmented eggs of *Bufo lentiginosus* are subjected to a temperature that

TABLE I.

No. of Exp.	Condition.	Temperature.	Time.	Result.
1	unsegmented.	28–30° C.	2½ hrs.	Normal development.
2	“	30–32	¾ “	Normal development.
2	“	“	1 “	Four eggs developed normally; the rest died or became abnormal.
2	“	“	2½ “	Most of the eggs died in the blastula stage; a few gastrulated but did not develop further.
3	“	32	2 “	All died in the blastula stage.
4	“	32–33	½ “	Normal development.
5	“	34–35	½ “	Irregular cleavage, no gastrulation.
5	“	“	1 “	Eggs killed.
6	2 cell.	31–33	1½ “	Normal development.
6	“	“	2 “	Most of the eggs developed normally.
7	“	35–36	¾ “	Abnormal cleavage, no gastrulation.
8	“	“	1 “	Development stopped at the eight-cell stage.
9	2–4 cell.	33–35	2 “	Abnormal cleavage, no gastrulation.
10	32–64 cell.	31–33	2 “	Normal development.
11	“	“	3 “	Four normal embryos; the rest of the eggs died or became very abnormal.
12	“	“	3½ “	All of the eggs became abnormal, none of them developed into tadpoles.
13	Late seg.	33–35	1½ “	Normal development.
13	“	“	2½ “	Normal development.
13	“	“	3½ “	Eggs killed.
14	“	36–37	½ “	Normal development.
14	“	“	¾ “	A few of the eggs developed normally, most of them died in the gastrula stage.
14	“	“	1 “	Eggs killed.
15	“	40–42	¼ “	Eggs killed.
16	Early gastrula.	33–35	3 “	Normal development.
17	“	35–37	1 “	Development stopped when the blastopore was closing in.
18	“	37–38	¼ “	Normal development.
18	“	“	1 “	Eggs killed.

stops their development before gastrulation begins, sections of the eggs show, in many cases, that the greatest injury has been produced in the yolk portion of the egg which is frequently vacuolated and not segmented although the upper part of the egg has divided into a large number of small cells. Hertwig has noticed the same phenomenon in some of his experiments, and in explanation he states as follows: “Dass Froscheier bei erhöhter Temperatur zunächst partiell geschädigt werden und eventuell absterben, ist offenbar auf die verschiedene Organisation der animalen und vegetativen Hälfte der Dotterkugel zurückzuführen. Die animale Hälfte der Dotterkugel ist reicher an Protoplasma und steht in höherem Masse unter der Herrschaft des Zellkerns. Unter der normalen Wechselwirkung von Protoplasma und Kern können aber Schäden, welche eine Zelle erlitten hat, wie durch verschiedene Experimente festgestellt worden ist, wieder rückgängig gemacht werden. In dieser Beziehung findet sich die vegetative Hälfte der Eikugel unter ungünstigeren Bedingungen. Denn hier ist das Protoplasma nicht nur spärlicher zwischen den Dotterplättchen vertheilt, sondern ist auch am ungethielten Ei mehr dem Einfluss des Zellkerns, der in der animalen Hälfte liegt, entrückt; später, nach Ablauf der ersten Furchungsstadien sind die Theilstücke vielmals grösser als die aus der animalen Eihälfte entstehenden Zellen."

When the injurious effects of the heat are not manifested until the eggs gastrulate, Hertwig (3) finds, in *Rana fusca*, that the abnormalities produced are of two sorts: First, those with a large yolk plug in the posterior region; second, those with deformed heads. In all of my experiments on *Bufo*, the abnormal tadpoles, with but very few exceptions, were of the first sort described by Hertwig. In some cases the development of the eggs stopped when the medullary folds were forming and a large yolk plug was found in the mid-dorsal region; in three cases only was the defect in the anterior part of the embryo. My results are more in accord with Hertwig's experiments on *Rana esculenta* than with those on *Rana fusca*, as in his experiments on the former species he obtained a much smaller number of spina bifida embryos than of those with a large yolk plug at the posterior end of the body.

Hertwig (4) finds that the optimum temperature for the devel opment of *Rana fusca* is 20° C. for the unsegmented egg, and that this optimum rises gradually to 24° C. for eggs in later stages of development. He adds: "Offenbar hängt diese Erscheinung damit zusammen, dass mit der Vermehung der Zellen die Kernsubstanz im Verhältniss zum Protoplasma immer mehr zunimmt und dass so das Protoplasma in höheren Masse ihrem Einfluss unterworfen ist." The optimum temperature for the unsegmented egg of *Bufo lentiginosus* is undoubtedly higher than that for *Rana fusca*, and it is probably somewhere near 28°

C. This optimum in increased 2–3° for eggs in later stages of development.

In another set of experiments on *Rana fusca*, Hertwig (4) finds that the maximum temperature to which the unsegmented eggs can be subjected without suffering any injury is 23–24° C., while this maximum is increased to 30° C. for eggs in the late segmentation stages. The maximum temperature for unsegmented eggs of *Rana esculenta* Hertwig finds to be 33° C. This is also the maximum temperature I have found for unsegmented eggs of the toad, although eggs in the blastula stage can endure a temperature of 38° C. for a very short time.

Morgan has noted that the blastula stages of *Rana palustris* can endure extreme cold much better than can eggs in the 2–4-cell stages, and he also finds that the eggs of *Rana temporaria* which are laid very early in the spring, can survive the tempera- of freezing water for several days. This temperature would very soon kill eggs of *Rana palustris* which are deposited much later than are the eggs of *Rana temporaria*.

While the eggs of all of these species of *Anura* can withstand a wide range of temperature without injury, there appears to be an adaptation to temperature corresponding to the different periods at which the eggs are deposited. *Rana fusca* and *Rana temporaria* lay their eggs very early in the spring when the water is often at the freezing point; and the eggs of these two species can stand cold much better than can the eggs of *Rana palustris* and *Rana esculenta* which are laid considerably later. Although the eggs of *Bufo lentiginosus* are laid but little later than are those of *Rana palustris*, they are usually deposited in shallow pools of water exposed to the direct rays of the sun. They must, therefore, often be subjected to a comparatively high degree of heat during the course of their development.

Bryn Mawr College,
Bryn Mawr, Pa., April 24, 1903.

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ON FLOSCULARIA CONKLINI, NOV. SPEC., WITH A KEY FOR THE IDENTIFICATION OF THE KNOWN SPECIES OF THE GENUS.

THOS. H. MONTGOMERY, JR.

1. FLOSCULARIA CONKLINI, nov. spec.

Corona with five lobes, the dorsal largest, the ventral next in size, the lateral very small. The lobes are broad, without knobs and confluent at their points of insertion upon the corona. Vibratile cilia, of a length not greater than that of the corona and sometimes considerably shorter, line these lobes in a single row, but are not present between the lobes. Corona usually less than half the length of the trunk, which is slender and not very sharply demarcated from the foot. Foot fully two and a half times the length of the rest of the body, terminating in a peduncle which is as broad as long. Dorsal and lateral sense organs are present, but no eyes in the adult. The body cavity is closely filled with numerous minute floating corpuscles, so that the animal appears dark by transmitted light. Tube large, gelatinous, usually with foreign particles adhering to its surface. Length about that of *F. campanulata* Dobie. Two or three ova are frequently found in the oviduct at once, and from thirty to forty male eggs within the tube.

This species I found in considerable numbers in a pond on the grounds of the University of Pennsylvania attached singly to *Myriophyllum*, during the early portion of 1903. It is a pleasure to me to name it in honor of my friend, Prof. Edwin G. Conklin. A full description of the anatomy with figures is reserved for another paper upon the morphology of the Flosculariidæ. The new form differs from the closely related *F. ambigua* Hudson in the shortness of the cilia and their vibratile nature (they are not stiff radiating setæ) in the much greater length of the foot and its very short peduncle, in the rather cylindrical and narrow corona, in its smaller size, and particularly in its germarium being rounded whereas in *ambigua* I have found it to be elongate and bent.

2. Key to the Species of Floscularia.

All the species of *Floscularia* (Oken, 1815) described up to the year 1886 are described and figured in the monograph of Hudson and Gosse. Of those described since that date I have seen the descriptions of all except *uniloba* Wierzejski (1892), so that this species could not be included in the present key. *F. brachyura* Barrois and Daday (1894) is considered unrecognizable: their figure represents a much contracted specimen, and the diagnosis is simply: "Pede rudimentario, in aculeo curvato exeunti, urcello nullo." But I differ from Rousselet (1893*b*) in regarding *tenuilobata* Anderson as distinct from *coronetta* Cubitt. *F. chimæra* Hudson is included, although it is probable this form will be subsequently found to belong to another family of the Rotatoria. Unless otherwise stated all the species entered will be understood to be sessile and to possess a gelatinous tube.

I. Foot ending in two toes (pelagic; no tube; 1 dorsal eye; corona with a smaller ventral and a larger dorsal lobe which overhangs the corona). *chimæra* Hudson (1889).
II. Foot without toes or peduncle (pelagic; foot very slender and whip-like; a single large dorsal coronal lobe and two smaller ventral lobes separated by a very slight constriction)............................*atrochoides* Wierzejski (1893).
III. Foot terminating in a peduncle.
  A. Corona without lobes.
    a, 1. Cilia short, in a single row (cilia mainly on dorsal and ventral margins of the corona; trunk much larger than corona and little shorter than foot; tube large)..*edentata* Collins (1872).
    a, 2. Short and vibratile cilia on outer coronal margin, and on 5 prominences of the inner margin longer, non-vibratile cilia (pelagic; tubes very slender). *pelagica* Rousselet (1893*a*).
  B. Coronal margin produced into lobes.
    a, 1. Short cuticular spines on coronal margin (corona with 5 broad lobes, the dorsal largest, all bearing long and stiff cilia)......*spinata* Hood (1893).
    a, 2. No cuticular spines on margin of the corona.
      b, 1. Corona with a single (dorsal) lobe (foot much enlarged near its posterior end; 2 eyes; a tuft of long cilia upon the dorsal lobe and shorter cilia upon the remaining margin of the corona; pelagic). *libera* Zacharias (1894).
      b, 2. Corona with two lobes, a dorsal and a ventral.
        c, 1. Lobes short, corona little wider than the trunk (short, non-vibratile cilia on the lobes only; 2 cervical eyes)......*calva* Hudson (1885).
        c, 2. Lobes large, corona much wider than the trunk (vibratile cilia on the whole margin of the corona; 2 eyes near the summit of the dorsal lobe) ....*mutabilis* Hudson (1885).
      b, 3. Corona with 3 lobes.

c, 1. Dorsal lobe with two long, flexible, non-ciliated processes (dorsal lobe much the largest, overarching the corona; short, vibratile cilia fringing the whole coronal margin in a double row; 2 eyes).
hoodii Hudson (1883).

c, 2. Dorsal lobe with 2 short, non-ciliated, horn-like processes on its dorsal surface (dorsal lobe largest, overarching the corona; entire margin of corona with an inner row of shorter and an outer row of longer cilia; no eyes)..*cucullata* Hood (1894).

c, 3. Dorsal lobe without any such processes.

d, 1. Lobes small, the dorsal one not overarching the corona.
gosseii Hood (1892*b*).

d, 2. Lobes large, the dorsal one overarching the corona.

e, 1. Lobes bearing cilia on their tips only (3 rings below the corona).
annulata Hood (1888).

e, 2. Entire margin of corona with a double row of cilia (inner row of short and outer of long cilia; lobes deeply marginate).
trilobata Collins (1872).

b, 4. Corona with 4 lobes (each bearing a tuft of very long cilia).
quadrilobata Hood (1892*a*).

b, 5. Corona with 5 lobes.

c, 1. Lobes very slender, longer than the whole trunk and nearly as long as the foot (with long cilia on their lateral borders).
millsii Kellicott (1885).

c, 2. Lobes shorter than the trunk.

d, 1. A flexible, slender, non-ciliated process on the dorsal lobe (lobes knobbed)..*cornuta* Dobie (1849).

d, 2. No such process on any of the lobes.

e, 1. Dorsal lobe trifid at the tip (dorsal lobe much the largest, the other lobes are slight projections of the coronal margin, and none with knobs; cilia rather short, limited to the lobes).
trifidlobata Pittock (1895).

e, 2. Dorsal lobe not trifid.

f, 1. Peduncle about one third the length of the extended foot, flexible (lobes not knobbed, rather pointed, the dorsal the largest and the lateral the smallest; cilia long, non-vibratile, along the whole coronal margin)......*longicaudata* Hudson (1883).

f, 2. Peduncle many times shorter than the foot.

g, 1. Lobes very slender, linear, composing almost the whole of the corona (lobes knobbed, with long cilia on their ends and short cilia elsewhere)...*tenuilobata* Anderson (1890).

g, 2. Lobes not linear, rest of corona distinct.

h, 1. Lobes knobbed.

i, 1. Cilia along the whole coronal margin.

j, 1. Lobes inserted on the coronal margin at some distance from each other (lobes very mobile and shorter than the diameter of the corona).
evansonii Anderson and Shephard (1892).

j, 2. Lobes confluent at their bases (fully as long as the diameter of the corona, not mobile; cilia long, non-vibratile; 2 eyes)...........*coronetta* Cubitt (1869).

i, 2. Cilia limited to the knobs of the lobes.
  j, 1. Cilia longer than the whole animal, extensile and very mobile..........................*mira* Hudson (1885).
  j, 2. Cilia not longer than the trunk, not clearly mobile.
    k, 1. Foot three times the length of the body (coronal lobes very short; 2 eyes)...*cyclops* Cubitt (1871).
    k, 2. Foot barely twicc the length of the body (coronal lobes well developed; no eyes). *ornata* Ehrenberg (1830).

h, 2. Lobes not knobbed.
  i, 1. Dorsal lobe overarching the corona so that its cilia point towards the foot (cilia non-vibratile, long). *torquilobata* Thorpe (1891).
  i, 2. Dorsal lobe not overarching the corona.
    j, 1. Cilia shorter than the corona, vibratile (cilia limited to the lobes; lateral lobes very small; the others somewhat triangular; corona usually less than half as long as the trunk; peduncle very short). *conklini* nov. spec.
    j, 2. Cilia longer than the corona, non-vibratile.
      k, 1. All five lobes distinct (corona as large as the trunk with cilia on its whole margin; peduncle long). *campanulata* Dobie (1849).
      k, 2. Lateral lobes small and indistinct.
        l, 1. Corona not ornamented with dots, tube distinct (germarium elongate, extending down the left side and across the whole diameter of the venter).................*ambigua* Hudson (1883).
        l, 2. Corona ornamented with dots in symmetrical patterns, apparently no tube (living within an algal growth)...........*algicola* Hudson (1886).

b, 6. Corona with 7 lobes.
  c, 1. Lobes not knobbed (long cilia around the whole margin of the corona). *diadema* Petr (1891).
  c, 2. Lobes knobbed (cilia restricted to these knobs; 2 eyes). *regalis* Hudson (1883).

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BIOLOGICAL BULLETIN.

FORM REGULATION IN CERIANTHUS.

I. The Typical Course of Regeneration.

C. M. CHILD.

Introduction.

During the year 1902–1903 it was my privilege to spend several months at the Zoölogical Station in Naples, as holder of the Smithsonian table. I take this opportunity to express my great indebtedness both to the Smithsonian Institution for the grant and to Professor Dohrn and all other members of the staff of the Zoölogical Station. A part of my time at Naples was devoted to the study of regeneration and other regulative processes in the Cerianthidæ, and an account of these observations and experiments is begun in the present paper.

So far as I am aware the only work upon regulation in *Cerianthus* is that of Loeb.[1] A review of this work is unnecessary at this time since the various points will be discussed in connection with my own observations as occasion arises.

My observations and experiments upon the Cerianthidæ fall into a number of groups, and, since they are somewhat extended, the account of the subject will be divided in a corresponding manner. In the present paper the usual "normal" course of regeneration resulting in a perfect animal is described. Later the problem of experimental control of regulation will be taken up, then variation and abnormalties in regulation and the factors concerned in their production.

The Normal Animal.

It is necessary to call attention to a number of the features of the normal anatomy and habits before proceeding to the description of the regenerative phenomena.

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[1] Loeb, J., "Untersuchungen zur Physiologischen Morphologie der Thiere," I., Wurzburg, 1891.

Cerianthus solitarius, the species which formed the subject of most of the experiments, is considerably smaller than *C. membranaceus*. Owing to the varying degrees of distension and contraction accurate measurements of the form are difficult to obtain. A considerable number of specimens were measured when apparently fully extended and the body distended with water. These were all among the larger specimens, for the smaller individuals were discarded in nearly all cases. These measurements are of course only approximate and serve merely to indicate the general proportions of the specimens used for experiment. Under other conditions of contraction or distension these same individuals possess very different proportions. In all cases a single individual was measured repeatedly at intervals and the maximum measurements taken as representing complete extension. The following table presents a few such measurements of different individuals, the measurements being given in millimeters :

Length of Body.	Length of Marginal Tentacles.	Length of Labial Tentacles.	Diameter of Disc.	Diameter of Body in Œsophageal Region.	Diameter of Body Near Aboral End
90	30–35	12–15	12	7	7
95	25–30	12–15	12	7	5
60	20	9–12	10	6	4.5

The specimens used were between these limits of size. A comparison of the measurements of the three individuals shows that the smaller specimen possesses different proportions from the larger, *i. e.*, its transverse diameters are relatively greater as compared with the length than those of the larger specimens. In other words, after the individuals reach a certain size further increase is chiefly an increase in length. Without giving the figures at this time to prove this point, since it will be taken up later in connection with the discussion of morphallaxis, it may be said that this difference in proportion between small and large specimens is of general and probably universal occurrence in *Cerianthus*. Smaller specimens are always relatively thicker than large ones.

In general form the body is nearly cylindrical, expanding orally to form the disc and tapering slightly posteriorly. At the aboral end is a small pore which under certain conditions permits the exit of water. In the expanded condition the disc possesses the form of a broad shallow funnel extending from the base of the marginal tentacles to the margin of the mouth and continued aborally in the œsophagus. The mouth is slit-like in form with one siphonoglyph or gonidial groove at one end of the slit. The disc is marked with radiating lines, slightly depressed, which correspond to the lines of attachment of the mesenteries beneath the surface: these continue aborally in the œsophagus. The œsophagus extends aborally from the disc about $\frac{1}{6}$–$\frac{1}{5}$ the length of the body when the animal is fully extended.

The marginal tentacles, as their name implies, are borne upon the margin of the disc, usually in about three rows, the number varying in grown specimens from about 41 to 71. About the margins of the mouth are the shorter labial tentacles which are fewer in number than the marginal tentacles, and form only a single circle.

The body appears brownish in color, but upon close examination is found to be marked with light longitudinal stripes or lines of varying width, some of which extend the whole length of the body while others are shorter. These are in reality merely unpigmented areas between the stripes of brown pigment. The color of the marginal tentacles is in general effect lighter than that of the body, but they are marked by transverse bands of dark pigment. The labial tentacles are brownish and usually unstriped. The disc and œsophagus in large, apparently old specimens are dark brown without definite striping.

As regards the internal anatomy certain points are of interest in this connection. It has long been known that the arrangement of the mesenteries in the Cerianthidæ differs in some respects from that in the other Actinozoa. In the œsophageal region all mesenteries extend from the body-wall to the œsophagus and thus divide the enteron of this region into a series of longitudinal radiating chambers which open into the enteron aborally. At the oral end each of these intermesenterial chambers opens into the cavity of a single marginal tentacle; thus the marginal tentacles are always equal in number to the intermesenterial chambers. The labial tentacles, while corresponding in position to intermesenterial chambers, are fewer in number.

Aboral to the œsophagus the inner margins of the mesenteries hang free in the enteron and bear the mesenterial filaments. A single pair of very short mesenteries at that end of the mouth where the siphonoglyph is situated are known as the directives. The next mesentery on each side of these extends almost to the aboral end of the body. From this point to right and left the mesenteries decrease in length, following a definite, rather complex law which need not be discussed here. On the side opposite the directives, at the opposite angle of the mouth are the shortest mesenteries, with the exception of the directives; these do not extend far aboral to the œsophagus. It is in this region that all new mesenteries are added, *i. e.*, the region of growth is opposite the directives. Thus, proceeding from the directives to the right and left around the body the mesenteries are successively younger. Each pair of new mesenteries appears between the members of the last preceding pair formed, thus separating them. Corresponding to the formation of new intermesenterial chambers new tentacles appear in this region. It is usually possible to find at this point in the normal animal one or two pairs of tentacles much smaller than the others and in process of growth. Corresponding to the number and arrangement of the mesenteries there is one unpaired marginal tentacle over the chamber between the directive mesenteries and known as the directive tentacle. It is usually somewhat thicker than the other tentacles since the space between the directives is greater than that between other mesenteries. The other tentacles are paired right and left.

In *Cerianthus solitarius* the greater number of the mesenteries about the whole circumference of the body do not extend aborally far beyond the œsophagus. Only certain mesenteries extend further, to end at various levels according to their position. This is also true of *Cerianthus membranaceus.*

The muscles of the body-wall consist of a heavy layer of powerful longitudinal muscles which decreases slightly in thickness toward the aboral end. These are the chief muscles of the body, circular muscles being absent, and tentacles, disc and œsophagus possessing only a slight muscular development.

As is well known, the Cerianthidæ are found imbedded in the sand with the oral end and tentacles protruding. In this position they secrete about the body a mass of tenacious slime in which sand-grains and other foreign bodies become imbedded, the whole forming a tube into which the animal may withdraw. Loeb has given an interesting account of the geotactic reactions of these animals and my incidental observations upon this point confirm his. He has also described a number of experiments concerning the external conditions which determine the tube-formation.

When specimens are kept in aquaria without sand they creep about to a considerable extent, often climbing the sides. When left undisturbed they usually orient themselves as Loeb has noted, so that the oral end of the body is directed upward, even if this position necessitates the bending of the body at right angles. In the jars they secrete a considerable amount of slime and often form tubes along the sides or bottom, in which they remain for a longer or shorter time. When handled or otherwise irritated, and especially when cut, the secretion of the slime is especially rapid.

When undisturbed, the body and tentacles are usually more or less distended with water and the body-wall is always tense. Indeed, as will be shown later, complete extension of the body and erection of the tentacles is impossible without internal water-pressure, *i. e.*, without water in the enteron. If the body of a distended animal is opened quickly by a small cut the water issues with considerable force, and when an individual is made to contract rapidly by sudden stimulation the water squirts from the aboral pore with great force. The inability of the animal to extend to its full length without the aid of water-pressure is due to the absence of circular muscles in the body-wall. Extension is passive, not active. The longitudinal muscles are powerful and under strong stimulus the body may be torn apart if the ends are fastened.

It was found that the animals could be kept alive for months without other food than the small forms and organic particles which the water might contain, and in the present series of experiments no attempt was made to give them food. Of course in the early stages of regeneration and throughout many of the experiments the pieces were unable to take food ; moreover, the growth resulting from an abundant food supply constitutes in any case a complicating factor in the analysis of various phenomena of form regulation. In such experiments as permit the taking of food a complete analysis of the phenomena would include studies of the effect of abundant food supply, but previous experiments along this line indicate that the results in the lower animals differ only in degree and not essentially in kind with the presence or absence of food.

Four species of *Cerianthus* were employed for experiment, viz., *C. solitarius*, *C. membranaceus* and two smaller undetermined species, one of them almost completely colorless. It was soon discovered, however, that *C. solitarius*, a very common form in the Bay of Naples, was more favorable than the other forms on account of size, coloration of body and abundance. My attention was therefore devoted chiefly to this species, though the other forms, and especially *C. membranaceus*, were used for comparative study.

THE COURSE OF REGENERATION.

The cut pieces were isolated in dishes which were placed in aquaria supplied with flowing water. During the earlier stages of regeneration the pieces showed little tendency to creep out of dishes, but later it was necessary in some cases to cover the dishes with netting to prevent escape.

In *Cerianthus* the course of regeneration is complicated in many cases by various factors, such as the form of the pieces, the internal water pressure, etc. The simplest cases are those in which the body is divided by a transverse cut into two pieces, or a piece is removed by two transverse cuts. In such cases a nearly cylindrical piece is obtained which regenerates at the cut end or ends. Such pieces are best fitted for the study of the "typical" course of regeneration at the two ends, and since a knowledge of this is important as a preliminary to the study of experimental control of regeneration this paper is devoted to a description of the phenomena concerned in such cases.

A piece cut from the middle region of the body (*e. g.*, between the lines *aa* and *bb*, Fig. 1) will serve as an example.

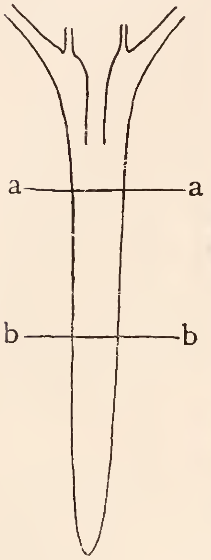
The Immediate Effects of the Operation.

Individuals which were in good condition and well extended were chosen and the cuts were made rapidly with sharp scissors. All parts of the body contract strongly in consequence of the cut, and of course total collapse of the piece occurs, owing to the escape of the water from the enteric cavity. Within a few moments the piece may relax somewhat from the extreme condition of contraction, but does not attain anything like its original length. Placed in the jar it lies on the bottom, and the weight of the tissues causes it to become more or less flattened. The piece has no power to retain its cylindrical form, though the mesenteries and mesenterial filaments, especially in pieces cut from the oral half of the body, partly fill the enteron and so cause the piece to retain a more or less rounded form. The body-wall is opaque in these pieces, while in normal specimens distended with water it is slightly translucent. The opacity is due simply to its greater thickness in the absence of the tension caused by internal water-pressure.

Within a few moments after section the cut edges at the two ends of the piece begin to bend or roll inward, and in an hour or two this inrolling has proceeded so far that the cut edges are no longer visible from the ends and the opening is almost completely closed by the inrolled portions. In Fig. 2 a longitudinal section through the oral end of such a piece is shown, the ectoderm and entoderm being indicated by full black lines and the thick muscular layer by fine lines. In this and following figures of the same kind the mesenteries are not shown; they of course occupy practically the whole of the enteric cavity after collapse. A section through the aboral end shows conditions similar to those figured in Fig. 2.

In consequence of the infolding about the whole circumference of the cut ends the circumference of the body-wall in the infolded region decreases greatly, although the transverse contraction of the body-wall during the infolding is not marked. It is, therefore, thrown into numerous longitudinal folds and ridges at the edge, and these appear when the piece is viewed from the end as folds and ridges radiating from what remains of the central opening.

Fig. 3[1] shows the oral end of a collapsed piece in which infolding has occurred. The numerous radiating foldings of the body wall are evident. Figs. 4 and 5 show the aboral ends of similar pieces. By this unfolding of the cut edges the openings at the ends of the piece are reduced to slits as is seen from the figures, and various parts of the circumference of the cut edge are approximated, though actual contact between parts of the cut edge cannot occur everywhere, owing to the irregular wrinkling of the margin as it folds inward. Indeed, since the margin does not contract transversely to any great extent as the infolding occurs, actual contact of all parts of the cut edge is a physical impossibility, as it could occur only by the reduction of the cut margin to a point at the center of the circle formed



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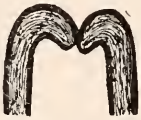

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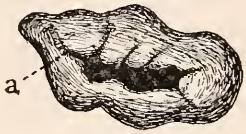

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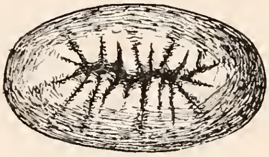

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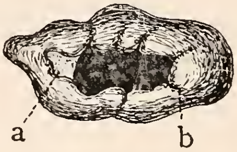

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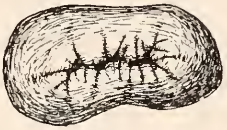

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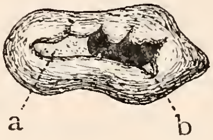

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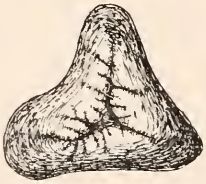

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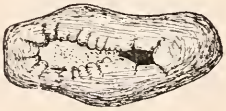

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[1] In Figs. 3, 4, 5, 6, 7, 8, 9, 10, 12 the longitudinal pigmentation of the body is not shown. The new tissue, where present, is indicated by stippling.

by the body-wall. In most cases, however, as the collapsed, more or less flattened piece lies on the bottom of the jar the infolding edges come into contact along the longer margins as in Figs. 3 and 4, leaving an elongated slit between them. In other cases the closure may occur as shown in Fig. 5. In general the form of the end depends wholly upon physical conditions and especially on the form of the transverse section of the piece after collapse.

The infolding of the cut margins is undoubtedly the result of mechanical conditions, though these conditions may themselves be in part reactive in nature. As Loeb has pointed out, an infolding must occur if the inner portions of the body-wall are under greater longitudinal tension than the outer portions. Such a condition may possibly be produced in the muscles near the cut, the inner layers undergoing greater contraction than the outer, but the elasticity of the fibrillar mesoglœa is probably in part responsible. As will be shown later this infolding produces in many cases conditions from which a return to the normal form is impossible. It can scarcely be regarded, therefore, as an adaptive reaction in the stricter sense. The radiating wrinkles and folds upon the end are due simply to the fact that the cut edges do not contract transversely as they are folded in.

As already noted, the result of the infolding is to close the terminal openings more or less completely. The closure is in no case perfect since between the irregular wrinkles there are always numerous interstices which afford communication between the enteron and the exterior. In most cases, however, these are soon blocked by the tenacious slime secreted by the ectoderm and are also frequently more or less completely filled by portions of the mesenteries or the filaments which happen to extend through them from within.

The Closure of the Ends.

The histological changes about the cut margins have not been fully investigated as yet, but it has been determined that growth of new tissue upon the edges begins soon after the cut is made. If after one or two days the infolded end be opened and carefully spread apart a very thin and delicate whitish membrane of new tissue will be found extending across parts of the opening. While growth undoubtedly begins on all parts of the cut surface, this membrane becomes distinct earlier at those regions where the cut edges are most closely approximated. Frequently when a piece is opened in the manner described the membrane will be found extending across regions corresponding to certain of the wrinkles about the opening but not yet covering the central area.

This method of formation of the thin membrane closing the end is well shown in a piece cut from a specimen of *C. membranaceus*. In this species the body-wall is so thick and stiff and the diameter of the body so great that in short pieces the infolding of the ends is often not sufficient to close the opening. In Fig. 6 a piece of this kind is shown. The new tissue first became evident along the fold *a*, and a day or two later a thin membrane was spread across this fold (Fig. 7, *a*. The new tissue is stippled). A little later still the fold at *b* (Fig. 7) also showed a thin membrane (Fig. 8), which, however, was afterward ruptured by contractions of the piece due to the stimulation incidental to examination. In Fig. 8 it is seen that the new tissue is gradually spreading over the opening from *a*. In Fig. 9 the opening is nearly closed. Several days later closure was complete. The changes in form of the piece as shown in the figures were the result of stimulation caused by the manipulation necessary for examination and drawing. In *C. solitarius* if the pieces are allowed to remain undisturbed at ordinary summer temperature the openings at the ends are usually completely closed by the thin membrane on the third day after operation. In the piece from *C. membranaceus* above described closure was complete after twenty-seven days. In general this species regenerates much more slowly than *C. solitarius*, but here the closure was exceptionally slow.

The membrane is easily ruptured by the contractions of the piece when strongly stimulated and great care is always necessary in the examination of such pieces to prevent rupture. In consequence of contraction the different parts of the margin change their relative positions or the mass of the mesenteries and filaments exerts pressure from within, thus readily causing rupture.

There is little difference as regards time of closure between the two ends, though in general the oral end is slightly in advance of the aboral end.

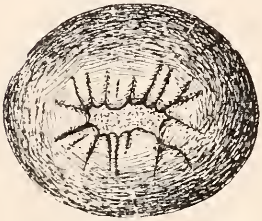
Distension of the Pieces with Water.

The piece remains completely collapsed during two or three days in summer and five to six days in winter, and then gradually becomes distended. At this time the piece is completely closed at both ends, no mouth or aboral pore being present. It is probable that the accumulation of water in the enteron is the result of diffusion through the walls, and especially through the very thin membranes at the two ends, in consequence of the accumulation of soluble products of metabolism in the closed enteron.

In the course of a day or two the piece becomes well filled with water and attains a degree of distension approaching that of the normal animal, though not as great. In some cases the accumulation of water in the enteron occurs so rapidly that the thin membranes closing the ends are ruptured and collapse occurs again, though usually the increase in thickness and strength of the membrane is sufficient to prevent rupture. The piece is usually well filled with water by the fourth day in summer and usually by the seventh or eighth in winter.

The immediate result of the renewed distension of the piece with water is, of course, the resumption of the cylindrical form ; the body wall becomes translucent and is elastic to the touch like that of the normal animal.

The most marked effect of the internal water pressure occurs, however, at the ends of the piece. So long as the piece remains collapsed the thin membrane closing the ends is not visible since the infolded edges of the body-wall are in close contact. As the body becomes distended with water, however, the infolded portions gradually spread apart and a central area covered by the new tissue becomes visible. Very small at first, it gradually increases in size until its diameter is about one third the diameter of the end. Fig. 10 shows the oral end of a piece at about this stage. The area within the folded margin of the old body-wall is covered by the thin membrane of new tissue. In Fig. 12 the aboral end of a similar piece is shown. There is little difference between the two ends, except that growth of the membrane is more rapid at the oral end. In Fig. 11[1] a portion of the oral end is shown more highly magnified. In this case the abrupt transition from the pigmented body-wall to the almost colorless new tissues is evident. From this figure it is also seen that the margin of the old body-wall is somewhat crenated by fine folds and wrinkles, which, however, are not regular in size and form, and do not represent the early stages of the new tentacles. The slight folds indicate more or less exactly the regions where the mesenteries are attached and the bulging areas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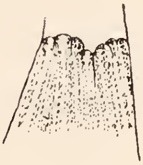
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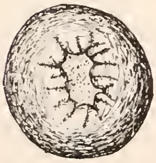
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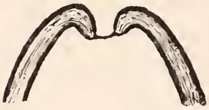
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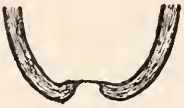
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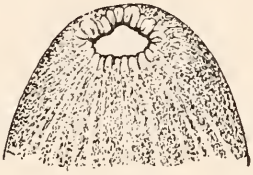
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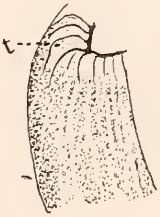

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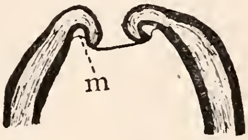

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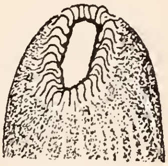
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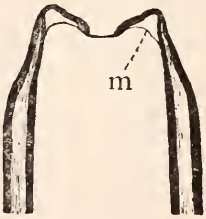

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[1] In Figs. 11, 15, 16, 18 the pigmentation of the body-wall is indicated.

between the intermesenterial chambers, these being now filled with water and under pressure. Here and there, however, folds without such significance occur, and moreover some of the chambers are so situated on the infolded margin that they are more widely open and thus expand more in consequence of the pressure than others, hence the irregularity in form and size of these crenations.

In Figs. 13 and 14 are shown respectively the oral and aboral ends of the body-wall at the stage where the infolded portions begin to separate. The thin membrane closing the end is shown as a black line. It consists, of course, of ectoderm and entoderm, but the muscular layer does not extend into it.

The Formation of the Marginal Tentacles and Disc.

Within the first day or two following the closure of the ends and the distension of the piece with water the changes leading to the formation of the characteristic organs of the oral end begin. In pieces cut from the middle region of the body the full number of mesenteries is not present, since some end anterior to this region. Regeneration of mesenteries occurs, though the number of mesenteries in a regenerated oral end from the middle region of the body is somewhat less than the number originally present at the oral end of the individual from which the piece is taken. This point will be considered at another time. It is sufficient for the present purpose to say that the whole oral end of the piece becomes divided into intermesenterial chambers, in the manner characteristic of the species, by the regeneration of new mesenteries, at first very short, between the longer mesenteries which are present in the piece. Attention was called above to the crenation of the infolded margin in correspondence with the position of the mesenteries (Fig. 11).

The first marked change following the closure of the end is the appearance of a slight ridge on the infolded margin of the old body-wall as shown in Fig. 15. The ridge is wholly confined to the tissue of the original body-wall, the thin membrane which closes the end playing no part in its formation. The crenations become more distinct and extend in many cases from the margin of the old body-wall over the ridge, as the regeneration of the mesenteries beneath advances. In Fig. 15 the ridge is shown as slightly lighter in color than the rest of the body. The pigmentation is beginning to disappear. Most of the stripes can still be followed over the ridge to the margin of the old tissue, but upon the ridge they are fainter than before. Fig. 16 shows a portion of the end at a slightly later stage, more highly magnified. Here the lighter color of the ridge is more distinct. While the body in general retains its brown color the ridge becomes light yellowish and its pigment disappears completely in the course of a day or two.

This change in pigmentation indicates that some alteration in the tissues is occurring, and the nature of the alteration becomes evident when a longitudinal section through the end (Fig. 17) is examined. This figure shows that the thickness of the body-wall and especially of the muscular layer is decreasing considerably in the region corresponding to the ridge. This decrease is shared to a certain extent by the ectoderm and entoderm as the figure indicates. The new regenerating mesenteries are minute folds in the infolded region, ending free aborally (*m*, Fig. 17).

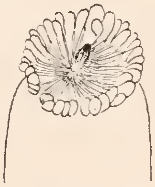
This ridge in which loss of pigmentation and reduction in thickness of the body-wall are taking place may be designated as the *marginal tentacular ridge*, since it is from this that the marginal tentacles arise; indeed the reduction in thickness of the body-wall and the division of the ridge into areas corresponding to the intermesenterial chambers are the preliminaries of tentacle formation.

The marginal tentacles do not arise from the cut edge of the body-wall itself but a short distance away from it, viz., at the highest point of the ridge (*t*, Fig. 16), *i. e.*, entirely within that portion which was originally part of the body-wall and not in the new tissue which closes the end.

Fig. 18 shows the oral end of a piece about a day later than the stage shown in Figs. 15 and 16. Here the new marginal tentacles are distinct and are evidently increasing in length. The pigment has disappeared completely from the tentacular ridge which is now whitish in color and distinctly translucent. Some of the tentacle buds are slightly broader than others owing to the fact that in the infolded condition of the margin some intermesenterial chambers were compressed and others stretched according to their position on the folds. There is, however, no marked difference in the length of the new tentacles on the different sides of the body, those in the region of the directives being no more advanced than those in the growing region opposite. From this figure it is very evident that the marginal tentacles arise from the highest, *i. e.*, the most oral point of the tentacular ridge. Moreover they arise in a single circle or row, although in the normal animal they occur in about three concentric circles.

A longitudinal section of the body-wall at this stage is shown in Fig. 19. A comparison with Fig. 18 shows marked changes. The most conspicuous of these is the continued reduction in thickness of the body-wall upon the ridge. The muscular layer has almost or quite disappeared in this region and also between it and the new tissue occupying the central region of the end, and is reduced considerably in thickness for some distance aboral to the ridge. At this stage then the whole oral end is closed by a thin, unpigmented, translucent membrane consisting of ectoderm and entoderm, but without a distinct muscular layer. The central part of this membrane resulted from the growth of new tissue at the cut edge, while the more distal portions forming the tentacular ridge have arisen by the transformation of a part of the old body-wall into tissue capable of a large amount of new growth, and of differentiation into new structures. In other words, the body-wall in this region has changed from its differentiated condition to what is commonly called the embryonic condition. The histological features of this change are of great interest, but will be described at another time.

The marginal tentacles now grow rapidly, and in another day (six days after the operation in summer) the oral end presents the appearance shown in Fig. 20. Several changes of importance have occurred since the stage of Fig. 18: the disc is greatly expanded, the marginal tentacles are much longer, the distinction between the tissue of the old body-wall and the thin membrane closing the end has disappeared completely, and finally the mouth is beginning to appear as an opening between the center and the periphery of the disc in the directive radius. The disc is marked with radiating lines, each of which terminates distally between the bases of two tentacles; those lines are in reality grooves and mark the lines of attachment of the mesenteries to the aboral surface of the disc. It will be seen that a small area in the center of the disc, indicated in the figure by stippling, is free from these lines; this represents that portion of the thin membrane beneath which regeneration of the mesenteries has not yet occurred. In the directive radius is situated a small opening, the new mouth, which gradually elongates in the directive plane.



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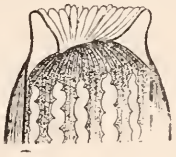

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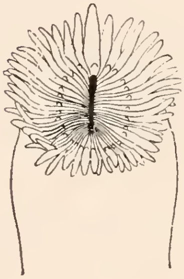

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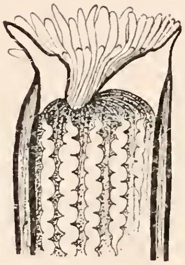

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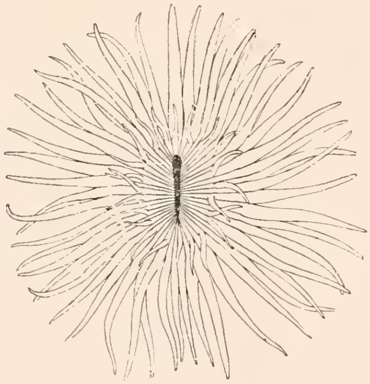

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The directive tentacle, which corresponds to the chamber between the two directive mesenteries, is slightly thicker than the other tentacles in consequence of the fact that the directive mesenteries are somewhat farther apart than other mesenteries. As regards the arrangement of the marginal tentacles it will be seen that they are no longer in a single row, but some appear as if they were being crowded out of the row, owing to lack of space. In all probability that is what is occurring. As the tentacles increase in size there is not sufficient space for them in a single row upon the margin and some are pushed out, probably in most cases peripherally.

Fig. 21 is a schematic figure of one half the body after longitudinal section in the directive plane, the directive tentacle being on the left of the figure. The stage of regeneration is about the same as that of Fig. 20. As compared with the earlier stages (*e. g.*, Fig. 19) several points of difference are to be noted: the marginal tentacles are longer, the difference in thickness between the reduced body wall of the tentacular region and the thin new tissue across the disc has completely disappeared; the reduction and disappearance of the muscular layer extends further aborally than before; the regeneration of the mesenteries has advanced; and finally there is a minute mouth, which, as was evident from Fig. 20, is not centrally placed, but lies near the base of the directive tentacle.

One or two points of importance as regards the regeneration of the mesenteries may be noted. In the normal animal a slight furrow, which appears as a faint longitudinal line on the surface of the entoderm, extends aborally from the aboral end of each mesentery. In a piece cut from the middle region many of the mesenteries lie wholly oral to the cut and so are not present in the piece, but most of the furrows, extending aborally, are visible in the piece. The mesenteries regenerate along these furrows. Whether regeneration of a particular mesentery aboral to the end of the furrow representing that mesentery is possible has not yet been determined. The point to which it is desired to call attention here is that the mesenterial regions are determined for some distance posterior to each of the mesenteries themselves.

Those mesenteries which extend into the piece undergo regressive changes, losing their thickened margins and filaments at the oral end, and become united with the new œsophagus.

THE APPEARANCE OF THE LABIAL TENTACLES AND THE LATER STAGES OF ORAL REGENERATION.

The marginal tentacles continue to increase rapidly in length and the œsophagus extends further across the disc from the directive side and also becomes deeper.

Fig. 22 is drawn from a stage three days later than Fig. 20 (nine days after the operation). Comparison of this figure with Fig. 20 shows at once the increased diameter of the disc, the greater length of the tentacles, and the marked change in the size and shape of the mouth opening. The tentacles in Fig. 22 are still of about equal size and length, except the directive tentacle, which is somewhat thicker and longer than the others. They still retain, to a large extent, the arrangement in a single row, though here and there a few have been forced out of line.

Upon the disc and forming a circle about the mouth appear the earliest stages of the labial tentacles. They are at this time mere buds, less than one half millimeter in length. All appear nearly simultaneously and develop with equal rapidity. As noted above, they are fewer in number than the marginal tentacles, some of the intermesenterial chambers possessing none.

A view of half the oral end at the stage of Fig. 22 after longitudinal section in the directive plane is shown in the schematic Fig. 23. In this case the plane of section passed through one of two small tentacles in the growing region opposite the directive tentacle; the section of this tentacle (on the right of the figure) is thus considerably smaller than that of the directive tentacle opposite. Comparison of Figs. 21 and 23 shows the changes which have occurred during the three days elapsing between the two stages. The œsophageal invagination is much deeper, the opening to the enteron is larger and the area of growing tissue, including the reduced body-wall, is much greater.

From this time on the course of regeneration consists in the gradual increase in size and the pigmentation of the regenerated parts in the manner characteristic of the species.

The problem of "morphallaxis," *i. e.*, the changes in the proportions of regenerating pieces leading to the more or less complete reëstablishment of the "normal" form will be considered elsewhere.

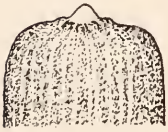
Fig. 24 shows a regenerated disc and tentacles at a later stage; in form and general arrangement of parts it is not distinguishable from the normal animal. The marginal tentacles have not yet fully attained their final arrangement; at present they are in two fairly well marked rows or circles. During the still later stages, however, as further increase in size occurs, the bases of some are forced still farther peripherally and so the characteristic arrangement of tentacles is finally acquired. The pigmentation of the marginal tentacles with dark transverse bands, which appears at this stage or earlier, is not shown in the figure.

The Differentiation of the Aboral End.

The infolding of the body-wall and the closure of the aboral end of a piece by a thin membrane have already been described. It remains to describe the formation of the characteristic aboral end. The course of regeneration here is much simpler than at the oral end.

The first marked change from the condition shown in Fig. 14 consists in the protrusion in conical form of the thin membrane closing the end (Fig. 25). About the margin of this new tissue the slightly wrinkled margins of the old body-wall are still clearly marked. In Fig. 26 a longitudinal section of the aboral end at this stage is shown. The absence of the aboral pore is to be noted. This new outgrowth at the aboral end does not become well-marked at once after closure, but only after the piece is well filled with water and the regeneration is advanced at the oral end, *i. e.*, it is much slower than oral regeneration.

In Fig. 27 the aboral outgrowth is seen at a somewhat more advanced stage. The wrinkles and folds upon the margin of the old tissue are gradually disappearing as this stretches and undergoes remoulding. A few days later the wrinkles have disappeared and there is no sharp distinction between the old body-wall and the new tissue at the time of union. Fig. 28 shows the end at this stage; and it is evident that the margins of the old body-wall are becoming involved in the regenerative changes in the same manner as at the oral end, for the pigment stripes are gradually fading out in the region which was before infolded. Fig. 29 shows a still later stage in which the gradual fading of the pigment-stripes is clearly seen. The significance of this loss pigment is made clear by Fig. 30, a longitudinal section of the aboral end at this stage. Here it is seen that a reduction of the muscular layer is occurring, *i. e.*, the old body-wall is becoming involved in the regulative changes for a short distance oral to the cut end: In other words the new aboral end is formed not merely from the new tissue which closes the end soon after operation, but, as in the regeneration of the oral end, in part from tissue derived from the margins of the body-wall near to the cut surface, by reduction of the muscular layer and growth of the ectoderm and entoderm. Thus the distinction between "old tissue" and "new tissue," at first well-marked, gradually disappears in this region.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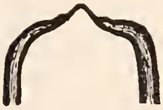
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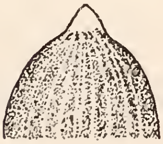
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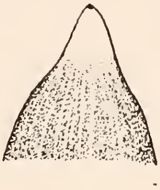
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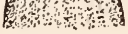
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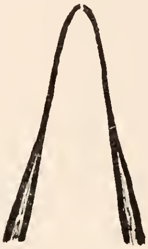
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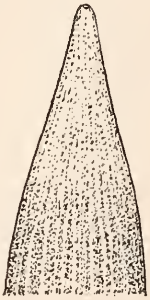
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Fig. 31 shows a still later stage in which the new tissue is becoming pigmented. The appearance of the pigment corresponds in time with the differentiation of the muscular layer, and I am inclined to believe that in *Cerianthus* as in various other forms, the pigmentation of the body is closely connected with the presence and arrangement of the muscular layer.

The course of regeneration described in the present paper is characteristic of pieces cut from the middle half of the body. In following papers the regeneration of pieces from various regions will be compared, and experiments determining some of the factors concerned in regeneration will be described.

SUMMARY.

1. In cylindrical pieces of *Cerianthus* obtained by two transverse cuts collapse occurs at once and the cut ends begin to roll inward soon after section, finally coming into contact and closing the opening more or less completely. Since little or no transverse contraction of the infolded margins occurs they are thrown into numerous radiating folds and wrinkles.

2. Within two to three days after section a thin membrane formed by the growth of new tissue from the cut surfaces closes the two ends completely. The piece now becomes gradually distended with water, probably owing to the accumulation of metabolic products in the enteron and consequent diffusion of water into this closed cavity. As distension proceeds the infolded margins of the body-wall at the two ends are forced apart by internal pressure and the area occupied by the thin membrane increases.

3. The first step in the regeneration of tentacles is the formation of a slight ridge, the marginal tentacular ridge, on the oral end. This ridge is formed wholly within the tissue of the old body-wall, its formation being accompanied by reduction and disappearance of the muscular layer, disappearance of the pigment and great reduction in thickness. The marginal tentacles first appear as slight upgrowths from the highest — most oral — point of the ridge, one tentacle corresponding to each intermesenterial chamber. The position of the mesenteries is indicated externally on the tentacular ridge by slight furrows which separate the regenerating tentacles from each other.

4. The regenerating marginal tentacles appear at first in a single circle and all usually regenerate with nearly equal rapidity, except in some cases the youngest pair in the growing region. The directive tentacle is usually slightly thicker than the others since the directive mesenteries are somewhat farther apart than the other mesenteries. Rapid increase in length occurs in the marginal tentacles, and the arrangement in about three circles or rows is gradually attained in consequence of the fact that there is not sufficient space on the margin of the disc for all of the tentacles in a single row ; some are forced peripherally by the mutual pressure exerted.

5. As the tentacles grow the disc expands and the distinction between the thin membrane of new tissue which first closed the end and the old body-wall with which it was connected disappears completely in consequence of the complete disappearance of the muscular layer, the reduction in thickness, and the loss of pigment in the body-wall of the oral end.

6. The mouth appears after the marginal tentacles are well established near the base of the directive tentacle, gradually extending along the directive plane across the center of the disc until it is symmetrical. The part of the mouth first regenerated is the region of the siphonoglyph.

7. The labial tentacles do not appear until the marginal tentacles have attained a length of several millimeters. Each tentacle appears as a distinct bud over an intermesenterial chamber, but some intermesenterial chambers are without labial tentacles.

8. After the aboral end is closed by the new tissue this slowly acquires a conical form, protruding from within the wrinkled margin of the old body-wall. The wrinkles on the latter gradually disappear and the pigmentation slowly fades out for a short distance oral to the cut end, this change being connected with reduction and disappearance of the muscular layer as this region of the body-wall becomes involved in processes of growth and redifferentiation in the same manner as the oral end. The aboral end grows out into an elongated conical form at the end of which the aboral pore appears. As the new muscles differentiate in this region pigment stripes begin to appear.

Hull Zoölogical Laboratory, University of Chicago,
July, 1903.

THE EYES OF THE BLIND VERTEBRATES OF NORTH AMERICA. VI.[1] THE EYES OF TYPHLOPS LUMBRICALIS (LINNÆUS), A BLIND SNAKE FROM CUBA.[2]

EFFA FUNK MUHSE.

Typhlops lumbricalis,[3] a blind snake, is generally distributed in the West Indies and Guiana. The specimens examined were obtained by Dr. C. H. Eigenmann in the neighborhood of Cañas, Province Pinar del Rio, Cuba. It is a burrowing form, that lives just beneath the surface, being thrown out even by the plow.

The snakes were first placed in formalin and after a few days were changed into alcohol. Only one young specimen was obtained, and it was preserved in Zenker's fluid. For decalcification, the heads of some were placed for at least three days in ten per cent. nitric acid and others in Perenyi's fluid from one to two weeks. One series was stained by the iron hæmatoxylin process, the others with hæmalum and eosin. It was very difficult to obtain satisfactory sections and especially complete series from the specimens, since no method was found to decalcify properly and to get the integument in condition for sectioning.

The lengths of the individuals examined were 10, 20, 21 and 21.5 cm. The color is brown above, on the ventral side it is yellowish-white. The body is covered with scales of uniform size, while those of the head are somewhat larger. The surface of the entire body is very smooth and shining and rather hard. The tail, which is about one twentieth of the body's length, ends in a short, sharp spine. The mouth is small and lies on the ventral side some distance back from the tip of the snout.

I. Normal Eyes of Snakes.

Snakes differ from other animals in having the edges of the two eyelids entirely grown together. A disk-shaped, conjunctival sac is thus formed and the layers over the eye between this sac and the exterior form the "brille."

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[1] Contributions from the Zoölogical Laboratory of Indiana University under the direction of C. H. Eigenmann.

[2] The blind vertebrates of Cuba are rated with those of North America.

[3] Boulenger, G. A., "Catalogue of the Snakes in the British Museum," 1893.

Six weakly developed muscles are present. The four straight ones arise in the neighborhood of the foramen opticus, while the two oblique ones arise from the surface of the prefrontal which is turned toward the eye socket.

Closely connected with the eye is a gland, Harder's, whose function is doubtful. Leading from this gland is a single duct, which either empties into the duct from Jacobson's gland or directly into the mouth cavity. The secretions of the gland are thus not functional in connection with the eye.

The sclera consists of closely woven fibers. Ciliary muscles are not found, but next to the iris is a great bundle of equatorial muscle fibers running obliquely, which seem to be a continuation of the iris musculature. The ciliary processes are weakly developed.

The retina consists of the usual layers. The nerve fiber layer is very thin (.003–.004 mm.).

The ganglion cell layer consists of a single, rarely two layers of small cells, each with a very large nucleus (.012–.013 mm.).

The inner reticular layer contains, at apparently regular intervals, elongated, oval nuclei (.042–.045 mm.).

The inner nuclear layer consists of two kinds of cells (.052–.054 mm.).

The outer reticular layer is very thin (.004–.005 mm.).

The sensory epithelium consists of the outer nuclear layer and the cone layer which is made up of single and twin cones. There are no rods. A single cone consists of two sections, an outer extremely small section, 5–6 microns in length and an inner much larger section, almost completely filled with a larger, pear-shaped, strongly refractive body, the ellipsoid, 14–16 microns in length and 8–9 microns across its widest part, which is turned toward the limiting membrane. The twin cone consists of two parts, one similar to a simple cone, the other cylindrical and very slender, its structure being otherwise like that of a simple cone. It is probable that the two parts of the twin cone are connected with but one nucleus. The nuclei of the cones vary greatly in form and leading from these into the inner layers of the retina are relatively very large fibers or processes.

Passing between the limiting membranes are the radial supporting Müllerian fibers.

II. THE EYE OF *Typhlops vermicularis.*

The work thus far on blind snakes has been done by Kohl on *Typhlops vermicularis*, a species found in Greece and the southwestern part of Asia, and on *Typhlops braminus*, a species found in the islands of the Indian Ocean and in Africa south of the equator, accounts of which are given in his "Rudimentäre Wirbelthieraugen."[1]

He found that in depth the eye of *Typhlops vermicularis* is equal to about one sixth that of *Tropidonotus.*

The brille is thicker in *Typhlops* than in *Tropidonotus* and, compared with the axial diameter of the respective eyes, is seven times thicker. In *Typhlops* the brille is equal in thickness to about one half that of the ordinary skin of the head. In *Tropidonotus* it is equal to one fourth.

The cornea of *Typhlops* measures .0052 mm., and compared with the relative sizes of the eyes is equal to about one half that of *Tropidonotus*, which measures .064 mm.

The conjunctiva is thickened at the edge of the disc-shaped sac and consists here of gland-cells, the fornix conjunctiva.

The supporting membranes of the eyeball, choroid and sclera are relatively equal to about one half those of *Tropidonotus.*

Harder's gland in *Typhlops* is many times larger than the eyeball.

The six muscles are present.

The lens is elliptical, while that of *Tropidonotus* is almost globular. The ratio of the lens volume of *Typhlops* to the eye volume is 1 : 14.04, while in *Tropidonotus* it is 1 : 3.6. The lens epithelium of the former is relatively six times greater than that of *Tropidonotus.*

The retina at the back of the eye of *Typhlops*, and the retina of *Tropidonotus* bear the actual ratio of 8 : 13, while compared with the eye axis in each case the *Typhlops*-retina is four times greater. The fovea centralis and area are absent.

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[1] Kohl, Dr. C. "Rudimentäre Wirbelthieraugen," Erster Thiel, Heft. 13, Bibliotheca Zoologica. Verlag von Theodor Fischer, 1892, Cassel.

The fiber layer has its greatest thickness near the exit of the nerve and gradually becomes thinner until, near the iris, scarcely a fiber is found.

The globular ganglion cells are arranged in a single layer except occasionally for short distances, when they lie in a double row.

The inner nuclear layer seems to be subdivided into four layers.

There are no twin cones. Each cone consists of a cone cell, stalk, middle and end members. The cone nuclei lie in two series, but the stalks vary in length so that the distal ends of the cone members reach nearly the same level.

III. The Eye of *Typhlops lumbricalis.*

The eye shows through the large ocular scale, which entirely covers it. It appears as a black spot surrounded by an unpigmented circle. The preocular, also a large scale, overlaps the ocular and reaches just to the edge of the eye (Figs. 1 and 2).

General Account of the Eye.

Compared with one of the garter snakes and in proportion to the size of the head, the eye of *Typhlops lumbricalis* is located further from the surface and occupies far less space, while Harder's gland, associated with the eye in both, is relatively much larger in *Typhlops.* In a specimen of *Typhlops lumbricalis* 21 cm. in length, the eye measured .306 mm. in width, and .387 mm. in depth. The greatest width of the gland of the same was .711 mm. and the length was 1.067 mm. The gland completely surrounds the eye up to the edges of the conjunctival sac (Figs. 3 and 4). In proportion to the size of the eyes, the gland of a garter snake is much smaller than that of *Typhlops lumbricalis*, but compared with *Rhineura floridana*[1] the gland of *Typhlops lumbricalis* is but little more than half as large.

The eye is covered by layers of epidermis and dermis, that differ from these same layers on neighboring parts by being thinner, more compact and free from pigment and glands. The ocular scale, however, which covers the eye region, does not differ in thickness from the other scales of the head (Fig. 3).

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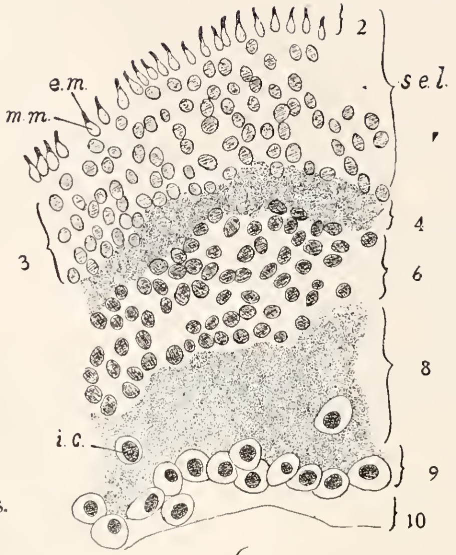
[1] Eigenmann, C. A., "The Eyes of *Rhineura floridana*," *Proceedings of the Washington Academy of Sciences*, Vol. IV., pp. 533–548, Sept. 30, 1902.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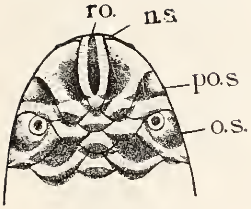
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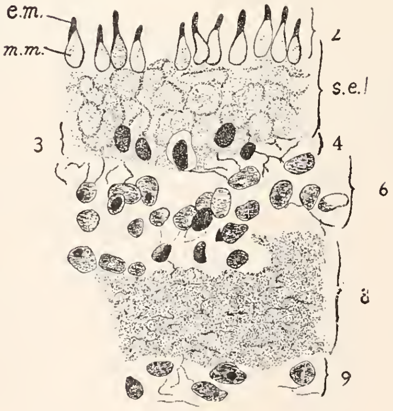
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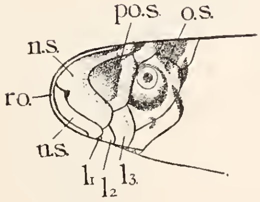
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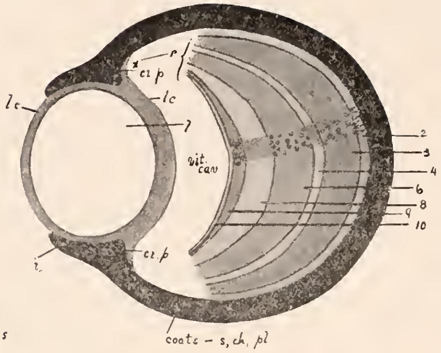
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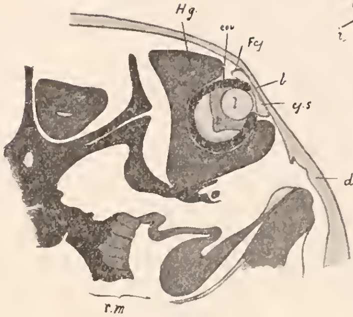
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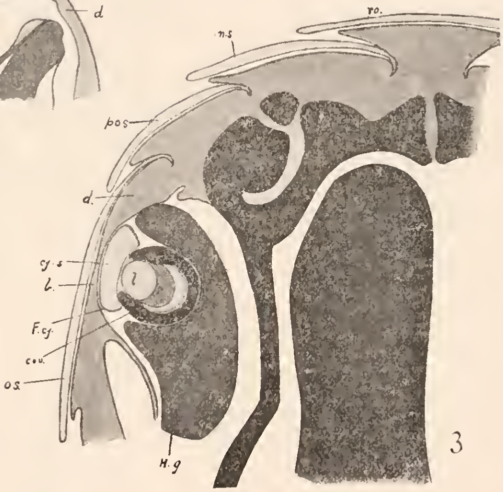
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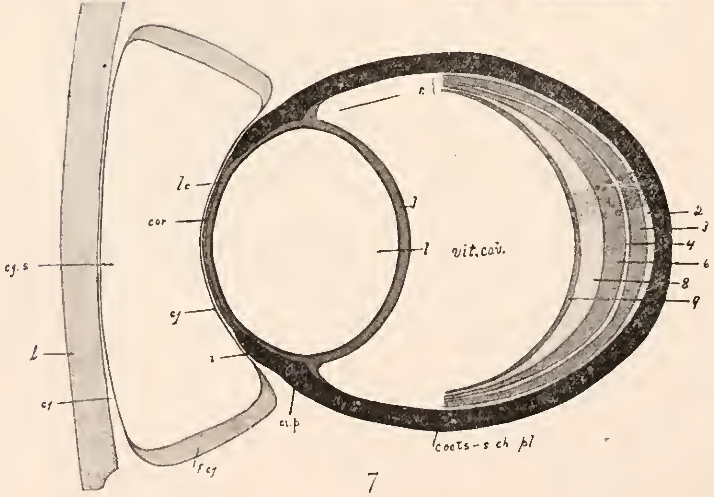
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A conjunctival sac is present with a diameter at least as great as the greatest width of the eye bulb. The conjunctiva, which forms this sac, is very thin over the cornea and next to the brille where it measures .003 mm. At the edge of the sac, it is differentiated into glands, the fornix conjunctiva, and measures .016 mm (Figs. 3 and 4).

In horizontal section, the eye axis is seen to be turned forward about 30° away from a line at right angle to the horizontal axis of the body.

Eye muscles are present, but from the sections used, the exact number could not be determined.

Minute Anatomy of Eye.

Choroid and Sclera.—The dense pigmentation makes it impossible to distinguish between the different coats at every point. Beyond the retina with its pigment layer is an open vascular space and this is followed by another dark layer, the two together representing the choroid. The choroidal pigmentary layer seems to consist of long fibers circularly arranged. The sclera can be followed by starting with the outer covering of the optic nerve and tracing its continuation about the eye.

Iris and Ciliary Processes. — Here again the pigmentation makes it difficult to determine the structure. Both iris and ciliary processes are present, for the black layer extends over the anterior surface of the lens, leaving a pupil equal in diameter to about one fourth of the circumference of the lens. At points near the equator of the lens this dark layer is enlarged into the ciliary processes and in connection with the capsule helps to hold the lens in place (Figs. 3 and 4).

Cornea. — This structure is present and can be traced to the region of the ciliary processes.

Lens. — A large lens is present, its depth being equal to about two fifths of the eye depth. From the sections little could be determined about its structure. A well-developed capsule surrounds it (Fig. 7).

Retina. — The same layers are present that are found in snakes in general, but the comparative thickness of the various layers is different. In the garter snakes, for instance, the retina is of a uniformly even thickness even to the ciliary process, a single layer of cells continues on over the surface of the processes and iris, but in *Typhlops lumbricalis* the retina at the back of the eye is very thick and gradually becomes thinner till it ends a short distance from the ciliary processes (Fig. 7). At this point the arrangement could not be definitely determined in the sections. At the back the retina, exclusive of the pigment layer, measures .0725 mm.

Ends of fibers were seen projecting inward from the ganglion cell layer, but no definite fiber layer could be distinguished (10 in Fig. 5).

The ganglion cell layer (9 in the figures) consists of a single row of large nucleated cells, somewhat irregularly arranged (.008 mm.).

The inner reticular layer (8) consists of a mass of fibers interwoven in a close network. This layer measures, at the back of the eye, .015 mm.

The inner nuclear layer (6) consists of at least three layers of cells, loosely arranged. The course of some of the fibers can be followed among these cells. This layer measures .016 mm.

The outer reticular layer (4) is very thin and consists of a few fibers so arranged as to leave a great number of spaces between the two nuclear layers. The distance between the nuclear layers is about .005 mm.

The sensory epithelium shows two distinct parts, an inner layer of nuclei (3) and an outer row of cones (2). In the sections these two were so separated that a loose tissue was visible, consisting probably of the limiting membrane and ends of the Müllerian fibers. The outer nuclear layer in the adult consists of a single row of nuclei, with a mass of quite homogeneous material about them. This part of the sensory epithelium measures .018 mm. The cones are pear-shaped bodies with the smaller end pointing outward, and at intervals of every four or five a shorter one occurs. Each element is differentiated into two parts. By the iron hæmatoxylin process of staining, the outer small end is densely stained, while the body of the element is a light granular mass (Fig. 5).

The pigment layer (1) is a continuous layer of even thickness, similar in every respect to that of the garter snake.

One young specimen, 10 cm. in length, was examined. The eye as a whole, as well as the lens, is nearly spherical. The eye measures in width .290 mm. and .322 mm. in depth. All parts are so developed that the vitreous cavity is relatively much smaller than that of the adult. The coats are thicker, the ciliary processes better developed, the lens capsule thicker, and the retina at the back actually measures one and two thirds the depth of the adult retina. The elements of each layer are much more numerous than in the adult, and they are packed much more closely together (Fig. 6). The ganglion nuclei are apparently arranged one against the other. In the inner reticular layer occur the "interpolated cells." These were not found in the sections of the adult eye that were examined. The cells of the inner nuclear layer are smaller and arranged in five or six rows. There is a well-developed outer reticular layer similar in its make-up to the inner reticular. Instead of a single row of cone nuclei with its surrounding homogeneous mass, as in the adult, this layer in the young consists of five or six rows of small, closely arranged cells. The cones likewise are smaller and more numerous (Fig. 6).

COMPARATIVE MEASUREMENTS OF RETINAL LAYERS IN MM.

	Fiber Layer.	Ganglion Cell Layer.	Inner Reticular Layer.	Inner Nuclear Layer.	Outer Reticular Layer.	Sensory Epithelium.	Total Depth.
Tropidonotus natrix.	.003	.012	.042	.052	.004	.0196	.1331
Typhlops vermicularis.	.0018	.0081	.0155	.0221	.0022	.0324	.0821
Typhlops lumbricalis (adult).		.008	.015	.016	.005	.030	.0725
Typhlops lumbricalis (young 10 cm.).	.005	.010	.024	.032	.008	.040	.1206

RELATIVE PROPORTIONS OF EYE PARTS.

	Tropidonotus natrix.	*Typhlops vermicularis.*	*Typhlops lumbricalis* (adult).
Eye depth.	2.5541 mm.	.4399 mm.	.4032 mm.
Brille :	Eye axis :: 1 :77.4	1 : 10.77	1 :12.5
Cornia :	Eye axis :: 1 :39.9	1 : 84.6	1 :85
Lens depth :	Eye axis :: 1 : 1.56	1 : 3.03	1 : 2.5
Coats :	Eye axis :: 1 :21.63	1 : 38.58	1 :25.4
Retina at back :	Eye axis :: 1 :19.19	1 : 5.36	1 : 5.5

EXPLANATION OF FIGURES.

Figs. 1 and 2 are from entire specimens. All figures except 1 and 2 are from sections. Figs. 7 and 8 are diagrams.

EXPLANATION OF NOTATIONS USED.

b. Brille.
ch. Choroid.
ci.p. Ciliary processes.
cj. Conjunctiva.
cj.s. Conjunctival sac.
cor. Cornea.
cov. Coverings of eye.
d. Dermis.
e.m. End member of cone.
F.cj. Fornix conjunctiva.
Hg. Harder's gland.
i. Iris.
i.c. Interpolated cells.
l. Lens.
l_1. First labial scale.
l_2. Second labial scale.
l_3. Third " " .
l.c. Lens capsule.
m.m. Middle member of cone.
n.s. Nasal scale.
o.c. Ocular scale.
p.l. Pigment layer.
po.s. Preocular scale.
r. Retina.
ro. Rostral.
r.m. Roof of mouth.
s. Sclera.
s.e.l. Sensory epithelium layer.
vit.cav. Vitreous cavity.

1. Pigment layer.
2. Cones.
3. Outer nuclear layer.
4. Outer reticular layer.
6. Inner nuclear layer.
8. Inner reticular layer.
9. Ganglion cell layer.
10. Fiber layer.

FIG. 1. Dorsal view of head of a specimen 21 cm. long.

FIG. 2. Lateral view of head of same specimen.

FIG. 3. Horizontal section of a specimen 20 cm. long, $2/3$-objective, 2-inch eye piece, camera lucida.

FIG. 4. Transverse section of a specimen 21 cm. long, $2/3$-objective, 2-inch eye piece, camera lucida. (Scales not shown.)

FIG. 5. Section of retina of an adult specimen 21 cm. long, $\frac{1}{12}$-objective, 1-inch eye piece, camera lucida.

FIG. 6. Section of retina of young specimen, 10 cm. long, $\frac{1}{12}$-objective, 1-inch eye piece, camera lucida.

FIG. 7. Diagrams of eye of adult.

FIG. 8. Diagram of eye of young.

(The region *x–r* in the sections could not be made out and is consequently left blank in the diagram.)

SOME EXPERIMENTS IN FEEDING LIZARDS WITH PROTECTIVELY COLORED INSECTS.[1]

ANNIE H. PRITCHETT.

During the past year, from October to May inclusive, I have been experimenting with insects that possess protective, mimetic and warning colors or that have some disagreeable characteristics which in a measure are supposed to prevent their being devoured by insect-eating animals. For this purpose several species of lizards found in the vicinity of Austin, Texas, have been kept in separate, convenient cages and fed with the various insects. Some interesting observations on the habits of the lizards were made incidentally and these are also noted in the following paper.

The species of lizards used for the experiments are the following: *Gerrhonotus infernalis* Baird, *Chrotaphytus collaris* Say, *Sceloporus floridanus* Baird, *Holbrookia texana* Troschel, *Cnemidophorus sexlineatus* Linn., *Phrynosoma cornutum* Harl., and an undetermined species of *Eumeces*.

EXPERIMENTS WITH SCELOPORUS FLORIDANUS.

LEPIDOPTERA.

Anosia plexippus Linn. This species is conspicuously colored in light brown with black and white markings. It is also said to have a disagreeable taste and is the supposed model of the mimic *Basilarchia disippus*. Specimens were introduced October 31, November 6, April 2 (two), April 4, April 6. Each time the butterfly was caught by the wing, or by the wings if folded, held for a few moments and then eaten slowly. It was not torn to pieces but held by part of the wings and swallowed gradually, the lizard often pausing a moment to rest.

Papilio (*Laërtias*) *philenor* Linn. Formerly this was included in the genus *Papilio* but has been separated because of characteristic differences, important among which is the supposition that it is an especially protected form because its larva feeds on *Aris-*

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[1] Contribution from the Zoölogical Laboratory of the University of Texas, No. 52.

tolochia, a poisonous plant of disagreeable taste. On October 30, March 27, March 30 (two), March 31, April 1 (three), April 4, April 16, April 23, May 4 (four), May 6 (two), butterflies were introduced into the cage and quickly eaten by the lizards with evident relish. On May 6 one of the specimens was badly mutilated and the lizards were not induced to take it for more than an hour.

Pieris occidentalis Reakirt. October 29, April 20 (three).

Pieris protodice Boisd-Lec. April 23, May 1 (four). These forms, white with black markings, were readily eaten.

Colias eurytheme Boisd. November 8 (two), March 31 (two), April 9, April 20 (seven), April 23 (five), May 1 (two). All quickly eaten.

Colias ariadne Edwards. April 16 (two).

Colias scuddere Reakirt. April 20, May 1 (two). These species are of striking yellow or orange marked with black, a typical warning combination, yet all were eaten eagerly.

Pyrrhanæa andria Scudder. This form is admirably protected by having the under side of the wings an exact imitation of a dead leaf. The wings are held folded closely together when the butterfly is at rest, and it remains motionless in this position for a great length of time. It is one of the most perfect instances of protective resemblance that I have obtained. Specimens were introduced November 14, April 22 (two, ♂ and ♀) and April 27. On April 22 the butterflies were not noticed at first. Several times they were offered to the lizards; the male was taken in about five minutes and the female ten minutes later. On April 27 the butterfly was seized by the wings several times, then dropped again. It remained motionless unless I moved it and the lizard would then seize it again. Finally it was abandoned, but it had disappeared the next day and probably had been eaten at last.

Pyrameis atalanta Linn. November 29. This is a conspicuous form, of black, brown, red and white. The lizards ate it eagerly.

Pyrameis huntera Fabr., a similar form but having large eye-spots underneath the wings. It was eaten May 1.

Grapta interrogationis Fabr. April 1. This species also has the under side of the wings in imitation of a dead leaf, and is very difficult to detect when at rest. It is in the habit of remaining motionless for a long while. The specimen introduced was at once eaten.

Papilio cresphontes Cramer. One specimen was introduced April 23 and four lizards at once seized the outspread wings. They showed no preference for the body but ate the wings first, as is usually the case. On May 7 the wings of the specimen introduced were almost entirely eaten when the lizard happened to drop it. It remained quiet, and the lizard would only take it again after I had made the butterfly move several times.[1]

Deilephila lineata. May 5. Two of these Sphingid moths were introduced and seized at once. They fluttered continuously and thus frustrated the attempts of several other lizards that were trying to participate. One moth was held by the head, the other by the wing for quite a while, till they ceased fluttering, and were then eaten.

Species unknown. May 4. This small moth is of black and orange, the typical warning coloration. It was eaten at once without any symptoms of dislike being shown.

HEMIPTERA.

Lygæid. May 5. Just after the above-mentioned moth was eaten four of these bugs were introduced. They are of the typical black-and-red or orange warning colors and have a very disagreeable odor. The same lizard that ate the moth at once seized a bug, chewed it a moment and spit it out, then licked his mouth for some time as if to remove the bad taste. Another lizard examined a second bug but made no attempt to take it. One bug was eaten later by the third lizard and the other two were gone next morning. May 13 a bug was introduced, seized at once and then rejected as before. It is evidently quite unpalatable.

Brachymena myops. Three were introduced November 8, but were never noticed by the lizards. The bug is gray in color, quite similar to the bark of trees that it frequents, and possesses a very unpleasant odor.

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[1] A glass jar containing live butterflies was placed on a chair about two and one half or three feet from the cage of *Sceloporus.* A large male lizard immediately climbed up the side of the cage, eyed the butterflies eagerly and seemed quite excited. This happened a few days later with several of the lizards. When the insects were introduced they were seized and eaten at once, several lizards quarreling over a desirable specimen and sharing it among themselves.

Fulgorid. Introduced November 5, November 6. This lantern fly is almost impossible to detect when at rest upon the trunks of the cedars and arbor-vitæ which it frequents. The upper wings and exposed portions of the head and thorax are somber gray, the almost transparent wings showing a tinge of pink when spread. The under wings are either entirely black or have a small white spot near the center. The posterior dorsal portion of the abdomen is bright red or deep orange, the remaining portions of the body being black. The insect shows perfect protective coloration at rest and a rather typical warning combination in flight. The insects were eaten at once by the lizards when seen in motion.

COLEOPTERA.

Chauliognathus scutellaris Lec. Although this beetle is colored black and yellow it appears to be palatable. May 1 five were introduced. The first was taken by the lizard that sampled the *Lygæid*, tasted a little, and rejected. However three others were eaten by a second lizard and the last beetle by a third. May 4 twenty beetles were introduced and all were eaten without any evidences of unpalatability. On May 5 four were introduced just after the four *Lygæids*. The first was carefully examined before being eaten ; the second was tasted and refused by another lizard ; the others were not noticed, as was also the case when seven were introduced the following day. The lizards were probably too well fed, for since then, May 11 and 13, they have eaten all that were offered.

Epicauta sp. November 3. This black blister-beetle was tasted and rejected immediately. Unfortunately no more specimens were found.

Zopherus haldemani Sallé. This very hard Tenebrionid beetle, conspicuously colored in black and white, was introduced November 9 and removed alive December 13 during which time no attempt to take it was seen. Specimens experimented with November 12 and May 5 gave the same results.

Lucanus dama Thumb. This black, horny beetle was introduced November 17 and died January 7; during this time the lizards never tried to take it.

Harpalus caliginosus Fab. This beetle is large, black and rather hard, nevertheless one was eaten December 2, one December 12 and another partly eaten January 8. Four remained dead at this date. Their odor is offensive.

Brachynus sp. When seized this beetle ejects a strong, volatile acid with a sharp, audible report. This always surprised the lizards; nevertheless, of the four beetles placed in the cage three were eaten, but the last refused. Two more were introduced February 26 and one March 5, which afterward disappeared and presumably were eaten.

Brachynus sp. April 3. This beetle, larger than the preceding species, was eaten at once.

Calosoma angulatus Chev. and

Pasimachus depressus Fab. were introduced March 17. The lizards attempted to catch them, but failed, and soon gave up the chase.

Chlænius orbus Horn. The odor of this beetle is quite offensive. March 9 one was eaten at once. On March 10 two lizards tried to catch a specimen but failed repeatedly. They appeared to notice the odor and gave up the chase. On March 23, however, the lizard that ate the former now ate another, and still a fourth was eaten April 3, but with evident disgust.

Cantharis fulvipennis Lec. This large blister beetle has the typical warning colors of black and yellowish-brown and is further protected by a disagreeable secretion that exudes from the joints of the legs when the insect is seized and which is capable of producing blisters. Four of these beetles were introduced May 19 and each was seized at once, then quickly shaken off. The lizards eyed the beetles intently, but made no attempts to take them. These specimens were removed and introduced again the following day. Only one beetle was taken this time and it was quickly rejected. On May 21 several beetles were again introduced. One was caught and quickly rejected and no further notice was taken of them unless they crawled upon the lizards, in which case they were shaken off violently.

DIPTERA.

Musca domestica and *Stomoxys calcitrans*. A small lizard of this species (*Sceloporus floridanus*) soon became so tame that it would lie on my hand and eat the flies which I caught and offered in my fingers. Sometimes he would catch the flies himself if I held him close to the window where they were crawling. He also ate a number of small spiders that were just emerging from the egg case placed in a glass jar. The lizard was kept in a cage with adults of the same species and was possibly eaten by them, as no trace of him could be found, and these lizards had, on two other occasions, been suspected of devouring small lizards.

HYMENOPTERA.

Pogonomyrmex barbatus var. *monefaciens.* These ants were eaten October 29, November 3, November 22 and May 24. The sting is quite severe.

Pachycondyla harpax, a stinging Ponerine ant, was eaten October 28.

Polistes annularis, a formidable wasp, was not noticed November 5.

ORTHOPTERA.

Gryllus abbreviatus. Several of these crickets were eaten March 7 and March 11. It is therefore probable that those introduced November 9, January 18, and January 19, were also eaten, since crickets seem to be a favorite food with all the species of lizards.

NEUROPTERA.

Panorpa nuptialis Gerst. This species has the wings of typical black and yellow warning colors. A female was introduced November 9 and a male November 15. Both disappeared in some way, but were not seen to be eaten.

ARACHNIDA.

Epeira fasciata Hentz. This protectively colored specimen was eaten October 25 and a second November 6.

SCORPIONS.

Centrurus caroliniensis Beauv. On March 23 the specimen which was introduced stung one of the lizards. He appeared to be in much pain and was so frightened at the scorpion that the experiment seemed likely to terminate there, but suddenly he seized the offending sting in his mouth and spitefully devoured the whole specimen. The color of this scorpion would seem to afford it efficient protection. This, together with its flat form, frequently prevents its being noticed by a casual observer when the stone under which it rests is overturned.

MYRIOPODA.

Julus (*Spirobolus*) *multistriatus* Walsh. The specimen introduced November 15 was not molested, but when two were introduced February 12 a lizard bit off part of the head of one *Julus*. Both specimens died after a few days, neither being eaten. This myriopod has a hard integument and is defended by means of an acrid secretion that is thrown out from the repugnatorial glands along each side of the body. It has the habit of coiling up and remaining quiescent whenever it is touched. This action makes the lizards suspicious of it.[1]

EXPERIMENTS WITH GERRHONOTUS INFERNALIS BAIRD.

The favorite foods of these lizards are crickets, grasshoppers, spiders and scorpions. A few Hemiptera were eaten also.

LEPIDOPTERA.

Anosia plexippus Linn. April 1, April 2, April 4 (three). None of these specimens were eaten.

Papilio (*Lærtias*) *philenor* Linn. March 26, March 30, April 6. All were examined and rejected.

Pyrameis cardui Linn. November 17. Offered and refused.

Pyrrhanæa andria Scudder. November 9. Refused.

Colias eurytheme Boisd. March 30, April 1 (three), April 6. On the latter date the butterfly was taken by the wings but soon dropped, and all others were refused entirely.

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[1] *Sceloporus floridanus* is badly infected with an interesting mite which attaches itself under the scales of the lizard until sexually mature and then crawls up on the wooden part of the cage to oviposit. The eggs are placed in a peculiarly constructed palisade and hatch as a six-legged larva that appears identical with the ordinary "red bug." The adult has a pubescent black integument; the head, anus and four pairs of legs are bright red. The legs are arranged in groups, two pairs being situated on the anterior portion of the body and two in the posterior region. Mr. Nathan Banks believes that this form may represent a new genus since it is the only lizard parasite that has been taken in this country, and appears to be closely related to the Italian genus *Geckobia*.

ORTHOPTERA.

Acridium americanum Scudd. November 15, November 24, January 28, March 11, March 30. This large grasshopper is of a very somber, dusty color and extremely quick in flight. Whenever introduced into the cage it was at once eaten eagerly. The lizard seized the insect by the thorax, held it thus for some time, regrasped it more anteriorly several times until the head was taken into the mouth. The insect was then swallowed slowly, the lizard chewing a while, pausing to rest, then gulping down another portion. On one occasion when the grasshopper became somewhat crooked, although it was nearly completely swallowed it was disgorged, straightened, and then devoured again.

Species unknown. On November 29 a large grasshopper was eaten in the usual way. The body, legs and head were dark green; the wings brown. The whole body was ornamented with white or yellow spots and lines.

Gryllus abbreviatus. December 12, January 10 (five), March 7 (several), March 10 (two), April 6 (several). All the specimens were eaten eagerly.

NEUROPTERA.

Panorpa nuptialis Gerst. November 9. Although this warningly-colored insect remained in the cage six days, no attempt was made to seize it.

COLEOPTERA.

Lucanus dama Thunb. November 8, was not eaten.

Zopherus haldemani Sallé. November 9, was refused.

Harpalus caliginosus Fab. December 11 and December 18. Five specimens were introduced, and all died.

Brachynus sp. February 12. Two of these beetles were introduced and were not noticed by the lizards, though offered repeatedly. They run very swiftly, hiding at every opportunity, and the lizards are probably too slow in their movements to catch so quick a prey.

Patrobus longicornis Say. The beetle was introduced February 13, and remained until March 5, but no attempt was made to take it.

Diabrotica punctata Oliv. February 13. These green-and-black beetles were probably too small for the lizards to perceive.

Chlænius orbus Horn. March 7. One of the lizards ran up to examine the beetle but when near turned aside, evidently discouraged because of the disagreeable odor, and did not try again to take it.

Pasimachus depressus Fab. March 17. The beetle was examined and refused.

Calosoma angulatus Chev. April 6. The beetle seemed never to have been noticed.

Chauliognathus scutellaris Lec. May 4. The lizards seemed to pay no attention to the beetle although fifteen specimens were introduced.

Cantharis fulvipennis Lec. Two specimens of this black-and-yellow blister beetle were introduced May 19. One was seized at once by one of the lizards, chewed a moment, then dropped quickly. The lizard began writhing and rubbing his mouth in the sand, appearing much distressed. The second beetle was not noticed by any of the lizards and was removed. On May 20 they eyed the beetle that was introduced, but made no attempt to take it. May 21, the specimen seemed not to be noticed. Others introduced May 26 gave the save negative result as the preceding experiment.

HEMIPTERA.

Brachymena myops. December 1, January 24. This protectively colored, malodorous form was not noticed by the lizards.

Lygæid. May 5. Two specimens of this warningly colored bug were introduced, examined and refused.

Fulgorid sp. November 5, November 6. Several specimens were eaten with evident relish. The bug was never refused if alive, but never eaten if dead.

HYMENOPTERA.

Polistes annularis. Linn. November 4, refused.

Camponotus sansabeanus Buckley. November 29 and *Camponotus festinatus* Buckley. April 13. These ants were possibly too small to be noticed.

ARACHNIDA.

Lathrodectes mactans. November 17, November 29, December 6 (two), December 18 (four), January 20 (two), February 2 (two), March 9, March 11, March 17, March 25, March 30, April 6, April 13 (four), April 20 (three), May 19 (two). These spiders are of a jet black color conspicuously marked with crimson or sometimes white, thus exhibiting striking warning coloration. They are even said to be poisonous, yet they were always quickly seized and eaten by these lizards.

Attus mystaceus. December 10, December 12 (two). The somber gray color of these spiders affords them good protection under the stones where they live. They were eaten eagerly.

Lycosa sp. March 9, March 23 (two), March 25. This spider resembles very closely in color the under side of the stones where it is often found. It was eaten at once when introduced.

SCORPIONS.

Centrurus caroliniensis Beauv. January 20, March 17, March 23 (two), April 13 (three), April 20 (six), April 27 (five), May 4, May 18 (six), May 19 (two), May 25 (two). All these specimens were eaten with evident relish and no attention was paid to the sting. The hard integument of the lizard prevents the penetration of the sting.

MYRIOPODA.

Julus (*Spirobolus*) *multistriatus* Walsh. The specimen was introduced November 18 and died January 7. It was not noticed by the lizards, as was also the case with two specimens introduced February 12.

EXPERIMENTS WITH CROTAPHYTUS COLLARIS Say.

Two of these lizards were captured November 9 and were not seen to eat a single insect until February 12. Various kinds of insects were placed in the cage, and though the lizards were quite tame and lively they would not eat. On January 23 a dish of water was placed in the cage and they learned to drink from the dish and also from the pipette used for refilling it. The water furnished their only nourishment for three months. A third lizard was captured April 4 and though very fierce at first, became quite tame in about a week, allowing me to rub its head and body with my hand. These lizards occupied the cage with *Gerrhonotus infernalis* until December 1 when they were placed in a separate one. The experiments were as follows :

LEPIDOPTERA.

Meganostoma eurydice Boisd. December 15, was not eaten.

Papilio (*Lærtias*) *philenor* Linn. March 26, March 30. These were not noticed and were afterward removed. The specimen introduced April 2 was found dead and apparently unharmed the following day. On April 7 the specimen introduced the previous day was gone, and on April 8 the lizard last caught was seen eating a butterfly. On April 21 a specimen was introduced and only a part of the wings remained next day. However, the two specimens introduced on May 4 remained in the cage two days and were not eaten.

Colias eurytheme Boisd. March 30. The specimen was not noticed by the lizards and was removed next day. The two that were introduced April 1 were gone the day following and of the two introduced April 2 one was entirely eaten and only the torn wings of the second remained. On May 8 one of the lizards seized the specimen just introduced by the edges of the folded wings and ate it slowly, often pausing to rest, but never releasing it.

Anosia plexippus Linn. Introduced April 2. Next day the head and thorax were chewed up and one fore wing was missing. Others that were introduced afterward disappeared but were not seen when eaten. But on May 18 the butterfly was seized at once by one of the lizards and a second lizard bit off part of a wing. Between them they ate the specimen, but did not take the two introduced May 25.

Pieris occidentalis Reakirt. April 16, was eaten.

Grapta interrogationis Fabr. The specimen introduced April 27 was eaten, and those placed in the cage May 7 and May 9 were gone the following mornings. Probably they were also eaten.

Papilio cresphontes Cramer. April 23. This butterfly did not seem to be noticed by the lizards.

Anosia berenice var. *strigosa* Bates. This butterfly has the same warning coloration scheme as *Anosia plexippus*. It had disappeared next day and was probably eaten.

COLEOPTERA.

The following specimens were introduced, but none of them were eaten and were rarely ever noticed by the lizards, though offered repeatedly :

Harpalus caliginosus Fab. December 2.

Brachynus sp. February 13 (three).

Chlænius orbus Horn. March 7, March 23, April 3.

Micryxis distinctus Hald. March 7. This beetle was evidently too small for the lizards to perceive. They pay no attention to small insects, possibly because their eyes are not capable of perceiving them.

Chauliognathus scutellaris Lec. May 4 (eighteen), May 5 (six). All refused.

A notable exception to this custom of refusing beetles was seen when three black-and-yellow blister beetles, *Cantharis fulvipennis* Lec., were introduced May 19. A lizard seized one of the beetles and ate it, then seized a second. One of the other lizards tried to take it from the former, but was unsuccessful, and the second beetle was eaten. The third was apparently not noticed by any of the lizards and was soon removed. Specimens were introduced May 20, May 21 and May 26, but did not seem to be noticed.

Occasionally larvæ of beetles were introduced and eaten, but with the above exception these lizards do not appear to feed on imaginal Coleoptera. *Cantharis* probably does not appear in the natural habitat of the lizard, the latter being a mountain species, while the beetle is found in the fields on the Mexican poppy (*Argemone mexicana*).

ORTHOPTERA.

Gryllus abbreviatus. February 12 three specimens were introduced, one of which was dead, and was at once seized and eaten by a lizard. This was the first food it had taken since its capture, November 9, and it is the only instance known of a lizard eating a dead insect. The two remaining crickets disappeared later and were evidently eaten. On March 23 one of the lizards tried repeatedly to catch one of the five crickets introduced, but failed, and finally gave up the chase, even refusing the insect when it was held before him in the forceps. The lizards were seen to catch and eat crickets on the following days: April 13 (two); April 20, April 27 (two), and on several occasions specimens that were introduced in the evening had disappeared by the following morning. Indeed, crickets seem to form the principal food of these lizards.

NEMOPTERA.

Panorpa nuptialis Gerst. December 12. This warningly-colored insect was apparently not noticed and died soon afterward.

DIPTERA.

Hermetia illucens Linn. December 13. This form resembles a wasp somewhat closely. It was not noticed by the lizards.

HEMIPTERA.

Lygæid sp. May 5. The lizards could not be induced to take the specimens.

HYMENOPTERA.

No experiments with Hymenoptera were made with these lizards.

ARACHNIDA.

Attus mystaceus. December 1. This spider was not noticed though offered repeatedly.

Lathrodectes mactans. Specimens were introduced January 20 (three), March 23 (two) but none were eaten.

Other small spiders (names unknown) were introduced at different times but were never eaten.

MYRIOPODA.

Scutigera forceps. December 18. Specimen refused.

SCORPIONS.

Centrurus caroliniensis Beauv. November 15. The scorpion stung one of the lizards and it seemed to suffer so intensely and was so frightened whenever the former came near it that the experiment was never repeated.

Three other species of lizards were placed in the same cage with *Crotaphytus collaris*, from which the following results were obtained:

1. *Cnemidophorus sexlineatus* Linn. One specimen was caught December 1 and died January 7 during which time it was never seen to take any food. This was also the case with two small lizards of this species that were in the cage with *Sceloporus*. They disappeared mysteriously and are supposed to have been devoured. The lizard is quite common, but difficult to catch, and it is regretted that more were not obtained for the experiments.

2. *Holbrookia texana* Trosch. Two of these lizards were placed in the cage early in April and have never been seen to take any food.

Eumeces sp. This small lizard was captured March 12. On March 30 it tore up and ate the body of a butterfly, *Pieris occidentalis* Reakirt. April 6 it caught, tore to pieces and ate a cricket larger in circumference than itself. April 8 it ate a large house fly and on April 10 a number of small mantids, *Stagmomantis carolina*, recently hatched. The lizard was very alert, spying the mantids at a distance of several inches, though the latter were quite small and exactly the color of the sand on the floor of the cage. On April 23 and May 8 other young mantids of the same size were eaten.

Phrynosoma cornutum Harl. The "horned toads" were kept in cages with other lizards and also separately and were never seen to eat anything but ants. They are especially fond of the large agricultural ant, *Pogonomyrmex barbatus* Smith var. *molefaciens* Buckley.

GENERAL SUMMARY.

1. Only one instance is known of a lizard eating a dead insect.

2. Insects that move slowly do not attract the attention of the lizards so much as do the more active forms, hence those that remain quiescent are rarely even attacked.

3. Insects below a certain size are apparently not perceived by the large species of lizards. Examples of such insects are *Diabrotica punctata* Oliv., *Micryxis distinctus* Hald., and various ants (*Camponotus*).

4. Large beetles having hard elytra are seldom eaten.

5. A butterfly with mutilated wings was not taken for an hour and a half although another perfect specimen introduced at the same time was eaten at once.

6. If an insect (*e. g.*, a beetle) falls upon its back the lizards rarely ever seize it until it has gotten upon its feet again.

7. The myriopod *Julus* was not eaten by any lizard.

8. Although the combinations of black and yellow, black and orange, or black and red are supposed to serve the purpose of warning coloration, all insects possessing these colors were, at one time or another, eaten, with the possible exception of *Panorpa nuptialis* Gerst and a malodorous *Lygæid* bug.

9. *Sceloporus floridanus* is perhaps the most satisfactory lizard for these experiments since it eats insects of all groups.

10. *Sceleporus* seizes any part of the insect, but as a rule only the wings of the butterflies and large moths.

11. All the lizards except *Eumeces* seize the insect with the mouth and swallow it a little at a time, never biting off pieces, but keeping the insect entire. *Eumeces* swallows its prey thus if small, but when the insect is large he shakes and pulls it to pieces with his mouth and eats the separate pieces.

12. *Sceloporus* is very active and is not easily tamed.

13. *Gerrhonotus* is exceedingly slow in capturing its prey. It creeps up stealthily, pauses when quite near, examines the insect by protruding the tongue, rises as high as possible on the toes of the fore limbs and then seizes the insect by the back with a sudden spring. If the insect does not move it is frequently left unmolested. This lizard soon becomes quite tame but does not enjoy being handled. It was seen to drink water from the dish by lapping with the tongue, but usually preferred taking it from the pipette, allowing me to place a drop at a time on its outstretched tongue.

14. *Eumeces* sometimes drinks by *lapping* with the tongue, sometimes by sucking up the water. *Sceloporus*, *Crotaphytus* and *Phrynosoma* drink by *sucking* the water into the mouth. At first *Sceloporus* and *Crotaphytus* would drink only from the pipette, but were gradually induced to follow that to the dish and drink from the latter.

15. *Phrynosoma cornutum*, though apparently quite tame, seems at first rather shy about eating in confinement. Ants, especially the agricultural ants (*Pogonomyrmex*), are its only known food.

16. *Crotaphytus* is not accurate in seizing its prey. It often fails repeatedly and gives up the attempt.

17. The larger lizards were several times suspected of having eaten smaller specimens that had been placed in the same cage.

18. *Crotaphytus* soon becomes quite tame and enjoys being petted. The smaller ones crawled upon my hand in the cage and refused to be put down.

19. The largest *Crotaphytus* shed its skin during the night of May 6. Next morning the sand in the cage was very much dug out and heaped up, but no traces of the skin could be found.

20. A *Gerrhonotus* shed during the night of April 29. The old skin was turned wrong side out and probably came off nearly whole, though several parts were broken when it was found next morning. A second lizard shed May 22 and I watched it pull the old skin off wrong side out by creeping round and round the cage close to the sides. The skin was loosened first from the upper and lower jaws along the sides of the mouth, and began to peel off backward by the lizard's rubbing its head against the sand on the bottom of the cage.

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The University of Texas, Zoölogical Laboratory,
Austin, Texas, June 4, 1903.

SEX RECOGNITION AMONG AMPHIPODS.[1]

S. J. HOLMES.

How do males of the amphipod crustacea distinguish the females? It is well known that the males of the Gammaridea have the curious habit of carrying the females under their body for a considerable time. This act of transportation has probably no further significance in relation to the fertilization of the eggs than to secure the proximity of the two sexes when the proper time for fertilization arrives. According to the observations of Della Valle on *Gammarus pungens* the eggs are not fertilized until after they are laid, oviposition occurring a short time after moulting. When the moulting of the female has been effected, the male bends his body beneath that of his mate and deposits spermatozoa upon the ventral surface of her thorax. The deposit of sperm is followed within half an hour by the laying of the eggs. After the act of copulation the male regains his original position and swims about with the female as before. The same relation of oviposition to moulting was found by Miss Langenbeck in *Microdeutopus*, the male leaving the female during her moulting process but soon resuming his previous position when the moult was completed.

The instinct of the male amphipod to seize and retain hold of the female is one of remarkable strength. The male retains his hold, despite all efforts to dislodge him, with remarkable persistence, and will still cling to the female after the posterior half of his body has been cut away. My own observations on the sexual behavior of amphipods relate mainly to three species, *Amphithoe longimana* Smith, *Hyalella dentata* Smith and *Gammarus fasciatus* Say. The sexual behavior of these three species is remarkably similar, athough they belong to as many distinct families. The female while being carried about keeps remarkably impassive. Her thoracic legs are drawn up, the abdomen held strongly flexed, the whole body assuming as compact a form as possible. She takes little or no part in swimming; the movement of the pleopods when the body is strongly bent upon itself serves only to keep a current of water passing by the gills. She is carried about like a helpless burden, allowing her vigorous spouse to assume the entire labor of transportation and the responsibility of keeping her as well as himself out of danger. The efforts of the male to seize the female and get her into the proper position to be carried have the effect of inducing her to throw herself into the characteristic bodily attitude and remain quiet. The attitude assumed by the female is similar to that observed in the ordinary thigmotactic reaction of amphipods and may, perhaps, be but the same form of response, somewhat modified and specialized in relation to the function of reproduction. When the males are torn away from the females they soon seize their partners again and roll them about into the proper position and then proceed on their way in apparent contentment. The female as soon as seized by the male curls up and allows herself to be rolled and tumbled about without a show of resistance or protest. The males, as a rule, are considerably larger than the females and usually get their partners into the desired position quite readily ; but when a small male attempts to carry a large female he experiences much difficulty. I have observed a male *Hyalella* endeavoring to carry a female somewhat larger than himself. After seizing the female he would turn her around until she finally came into the proper position for transportation, but owing to the larger size of his partner the male could not reach around her body so as to carry her away. No sooner was the female properly adjusted than the male would lose hold of her round body and the same efforts had to be repeated. During all this performance the female remained dutifully passive. After watching the further struggles of the male for over half an hour I became convinced, although he was not, that he had undertaken an impossible task, and discontinued my observations.

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[1] From the Zoölogical Laboratory of the University of Michigan, Ann Arbor, Mich.

In order to ascertain if sight plays any part in sex recognition in *Hyalella*, I tore some males away from their partners, blackened their eyes with asphalt varnish, and placed them in a dish with several females. It was not long before each of the blinded males was provided with a mate. Sight, therefore, is not the determining factor in sex recognition in this species.

That the females are distinguished through the sense of smell seemed more probable, since it has been shown that among many insects sex recognition is brought about in this way. The sense of smell in crustaceans is often highly developed and in some groups probably affords the means by which the females are distinguished. The sense of smell in the crustacea is mainly, although not quite exclusively,[1] located in the first antennæ. To determine if the male distinguishes the other sex by this sense, recourse was had to the experiment which naturally suggested itself, of removing the first antennæ of several males and observing whether they experienced any difficulty in finding mates. It was found that after they had recovered from the slight shock of the operation, the males seized the females as eagerly as before and carried them about in the usual manner. Even after both pairs of antennæ were removed the females were seized and carried in the same way. It is very improbable, therefore, that the sense of smell plays an important part in enabling male Hyalellas to distinguish the other sex. The experiment was then tried of placing several females in a small enclosure of wire gauze, while several males which had recently been torn from females were placed in the same dish, but outside of the enclosure. The males paid not the slightest attention to the females within the gauze ; but soon after the gauze was raised and the females allowed to scatter through the dish most of the males had acquired a partner.

If one attentively observes Hyalellas as they are swimming about, it will be seen that the males do not pursue the females, great as their eagerness may be to seize and carry one of the opposite sex. Only when the two sexes collide in their apparently random movements does the male become aware of the presence of the female. When a male and a female collide, the female curls up and lies quiet while the male makes efforts to seize her. Should two females collide, they may curl up for a moment, but as they are not seized they soon pass on. When two males meet there is often a lively struggle. Each apparently attempts to seize and carry the other, but as neither will consent to remain passive they soon separate. The different reactions of the two sexes to contact with other individuals is the factor which effects the union of the males with the females. Each reacts to the reactions of the other. The male has a strong instinct to seize and carry other individuals of the same species. The female has the instinct to lie quiet when another individual comes into contact with her, especially if she is seized. The instinctive reactions of the two sexes are complementary and coöperate to bring about and maintain the peculiar sexual association characteristic of the Gammaridea. If the association of the sexes is brought about by their peculiar modes of reaction to certain contact stimuli, it would seem probable that the only reason why males do not carry other males as well as females is that they are prevented from so doing by the active resistance of their intended mates. I was accordingly led to try the experiment of mutilating some male specimens so that they could no longer make effective resistance to seizure. The large second gnathopods (the principal means of defense) of several males were cut off and the mutilated individuals were placed in a dish with several males which were recently torn from females. The mutilated males were soon seized and carried about as if they were members of the other sex. In one case a mutilated male was carried about for over five hours. The mutilated males were more active than females are under the same conditions, and did not assume the same bodily attitude, but nevertheless their captors carried them without any manifest awareness of the deception to which they were subjected.

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[1] Bethe, *Archiv. mic. Anat.*, Bd. 2, 1897 ; Holmes, BIOL. BULL., Vol. II., 1901.

Male Hyalellas, however, will not carry dead specimens of either sex, at least for more than a short time. I have observed males of both *Hyalella* and *Gammarus* struggling for a time with a dead specimen, but their efforts to carry it were soon discontinued. The failure to carry dead individuals may be due to odor or some sort of chemical stimulation from the object seized, or to the lack of an occasional movement causing a struggle on the part of the male to retain his hold. Stimuli of the latter kind may be necessary to cause the instinctive reaction of the male to continue.

There can be little doubt that the origin of the instinct of the male amphipod to seize and carry the female is to be sought in a modification of the act of copulation. The lower crustacea afford many cases in which the association of the two sexes is prolonged for a considerable period. The males of *Artemia* clasp the females with their peculiarly modified antennæ and the two sexes swim about together for several days (Leydig). Among the free-swimming copepods the male may continue clasping the female for some hours after, as well as before, depositing the spermatophore (Jurine, von Siebold). And among the Cumacea Dohrn has observed the males swimming about upon the backs of the females, much as in Amphipoda. The tendency for the association of the sexes greatly to exceed the act of copulation is apparently quite widespread among the crustacea ; and although, so far as is known, the mating instinct of the Gammaridea is much the same throughout the group so that we cannot trace the successive steps in its development, the sexual behavior of some of the lower crustacea presents many features which may serve to throw some light upon its origin.

REGENERATION OF THE LEG OF AMPHIUMA MEANS.

T. H. MORGAN.

My object in studying the regeneration of the limbs of *Amphiuma means* was to discover whether the limbs, which appear to be of so little use to the animal as organs of locomotion, have the power to regenerate as have the limbs of other urodele amphibia.

The first amphiuma that I obtained (in 1900) was a large individual, and after several months had begun to regenerate, but died as the result of an accident before regeneration had gone very far.[1] The next individual that I was able to procure was also large, but escaped before regeneration had gone any farther than in the last case. Two smaller individuals have been kept for more than a year (from March 21, 1901, to May 3, 1902). The following account applies to them. Each had a fore-leg and hind-leg of opposite sides cut off through the upper portion of the leg. In the course of several weeks a knob of new tissue appeared which continued to elongate for several months, when further growth seemed to have ceased. To make certain of this, the animals were kept for six months longer, but no further change occurred. The new part was shorter than the part removed, and appeared to be a single rod, tapering at the end, without any external signs of toes.

The normal fore- and hind-foot of the amphiumas that I used had each three toes. Cope[2] gives a figure of the skeleton of amphiuma showing a cartilaginous carpus of four or five pieces, and three ossified metacarpals with ossified phalanges. In the hind-foot there are three cartilaginous tarsalia, three ossified metatarsals and three phalanges.

After the legs had regenerated they were cut off, imbedded in paraffine, and cut into sections. These showed in three of the four cases that the two bones of the middle part of the limb have developed. The condition of the carpus and tarsus appears to be different in each of the four cases, Figs. 1–4. The rough reconstructions shown in these figures were made from sections. The figures are not very accurate, but serve to show the number of bones and their relation to each other. The relative sizes of the bones is less exact. It will be seen from the figures that the regeneration has lead neither to the formation of a uniserial row of skeletal elements, nor is it clear in all cases whether more than a single toe is represented. It seems probable that the terminal middle phalanx represents a toe, but whether any of the other cartilages represent other suppressed toes can not be stated.

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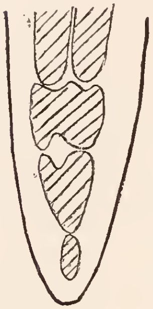
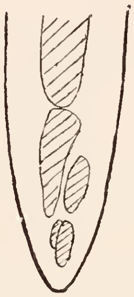
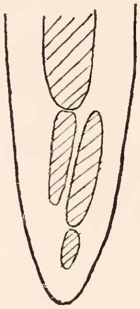
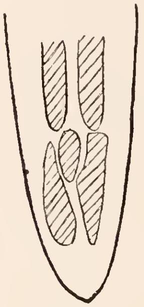
[1] This is the case referred to in Towle's paper. BIOLOGICAL BULLETIN, II., 1901.

[2] Cope, "The Batrachia of North America," Bull. U. S. Nat. Mus. No. 34.









In these four cases the legs had been cut off through the humerus, or the femur. It occurred to me that if the limb were cut off through the fore-arm or the fore-leg the result might possibly be different, since two bones are present at the cut surface. Therefore on May 3, 1902, when the two regenerated legs were removed for study, the remaining two legs were cut off through the fore-leg and fore-arm.

The two amphiuma were kept alive for nearly another year; until March 30, 1903. They were occasionally fed on earthworms. The limbs that had been cut off through the fore-arm and fore-leg regenerated, but again produced only a single pointed, or in one case a somewhat flattened, new part. Serial sections show that, besides completing the ends of the two bones at the exposed surface, there have been produced a number of more distal cartilages. The arrangement of these pieces is irregular, and different in each case, as also occurred when the leg was cut off through the upper portion. In other words, no better regeneration took place here than in the former instances.

It is also of interest to notice that the other two legs that had been cut off (close to the body) for examination had not regenerated. The skin grew over the cut surface, and in several cases the muscles of the body wall seemed to have grown over the short piece of the humerus or femur that had been left. At most, a short protrusion indicated the position of the limb.

How shall we interpret this result. Those who hold that the power to regenerate a part is commensurate with the value of the part to the animal, if it is a part liable to injury, will welcome this experiment as in harmony with their interpretation. On the other hand, as I have tried to show elsewhere, the evidence is so strong against this point of view that I think we shall not go wrong if in this case we deny that the result has any such meaning.

In fact, in other adult amphibia, in the frogs for instance, in which the limbs are of some importance to the animal they cannot be regenerated, although in the tadpole stage in which the limbs are of no importance, and, in the case of the fore-limb at least, not liable to injury, the power of regeneration is present. Moreover even in the urodeles the power of regeneration is unequally developed in forms that use their legs for purposes of locomotion. It is said that *Triton marmoratus* shows only a slight power to regenerate its legs. In other cases, as I have observed in *Necturus*, the time required to regenerate a leg is so long that were the presence of the leg essential to the existence of the individual it would succumb before the regeneration could take place.

These considerations make it clear, in my opinion, that the lack of complete power to regenerate in amphiuma can not be interpreted as having any connection with the unimportance of the legs to the animal. It should not be overlooked that it is not that the leg does not regenerate at all ; in fact it regenerates quite well, but that the new part is different from the old. It is at least conceivable that some simple physical or physiological factor may interfere with the formation of the complete toes, such, for instance, as the thickness of the skin in relation to the size of the limb.

If it could be shown that the leg of amphiuma is a degenerate structure it might appear that there is some connection between the degeneracy of the part and its lack of power to regenerate, but it is far from being established that any such general relation really exists. In fact, in the male hermit crab I found that the very small and *apparently* rudimentary abdominal appendages have the power to regenerate. It would be interesting, nevertheless, to examine this point further in cases where the degeneration and uselessness of an organ are more certainly established, as in the case, for example, of the appendix of man, which does not appear to have the power to regenerate after removal.

WOODS HOLL, MASS., June 22, 1903.

Vol. V. *November, 1903.* *No. 6*

BIOLOGICAL BULLETIN.

ABSORPTION OF THE HYDRANTH IN HYDROID POLYPS.

H. F. THACHER.

In 1900 there appeared a paper[1] by Professor Loeb on the "Transformation and Regeneration of Organs," the first part of which contained a discussion of the process of absorption in campanularia hydroids. His results were obtained from a study of the effects produced on the polyps by placing them in shallow dishes of sea water, so that they were in contact with the glass; under these conditions he found that they were gradually transformed and at length absorbed completely into the stem. To summarize briefly Loeb's account of this process, he states that there is noticeable first a contraction of the animal into the cup, followed by the fusion of the tentacles and later by the withdrawal of the whole polyp — now a shapeless mass of protoplasm — into the stem. This complete transformation he ascribes to contact, since it "is certain that contact with sea-water favors the formation of polyps with their more solid elements, while the contact with solid bodies favors the formation of the more fluid material of the stem or stolon." It seemed probable that a histological examination of these changes, in which the hydroid is represented as transforming and *creeping back into the stem*, might prove of interest, since they involved a complete transformation of well-differentiated structures. Therefore, at Professor Morgan's suggestion, I worked on this subject at Woods Holl during the summer of 1902. I was able to obtain a table first through the kindness of the director, and later was appointed to the Bryn Mawr table.

On examining the literature it will be found that there are frequent references to the absorption or disappearance of polyps. Loeb finds for *Margelis* and *Antennularia* that the polyps

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[1] *The American Journal of Physiology*, IV., 1900.

"disappear" when their condition of growth is disturbed — *i. e.*, the former being brought into contact with a solid, the latter being suspended horizontally so that its relation to gravity is changed. *Eudendrium*, according to some workers, sheds its hydranths when brought into the laboratory, but I have also often found absorption occurring under the same conditions, and *Eudendrium tenue*, a smaller and more delicate form than *Eudendrium racemosum*, responds in this way even more constantly. *Pennaria*[1] has recently been examined by Cerfontaine who finds that the day after the hydroids have been collected "ca matérialse trourait dans un mauvais état, les polypes qui persistaient étaient morts, les parties mollas s'étaient retireés dans la perisarque et les extremitées du cœnosarque réduit s'étaient cicatricées. Si l'ou conserve les branches, en maintenant une circulation d'eau de mer, ou les voit souvent reprendre de la vigueur. . . . Ou peut de cette façon déterminer expérimentalement une repetition de la regeneration spontanée. A la suite des troubles brusques produits dans les conditions d'être de ces organismes, par la récolte, le transport, le changement d'eau, le changement de température, de lumière, etc., ou determine rapidement la destruction des polypes ; mais bientôt, il semple se produire une acclimation rapide, et aussitôt une nouvelle régénération commence." *Tubularia* never absorbs its polyps but sheds them soon after being collected, and after a day or so if undisturbed, new polyps grow out from the old stalk, a new growth of stalk also taking place behind the head.

It seemed possible that the absorption of the heads of *Campanularia* might be analogous to that in these other forms, in which case it should occur even when not in contact with solids. To test this, I left the hydroids still growing on bits of wood, and placed them in the dishes, so that they were completely surrounded by water. Nevertheless the polyps began to absorb and by the end of twelve hours had almost entirely disappeared, while a few new ones were beginning to form from the old stalks. I also noticed on examining dishes of unused hydroids that had been standing over night, a large percentage of absorbing polyps.

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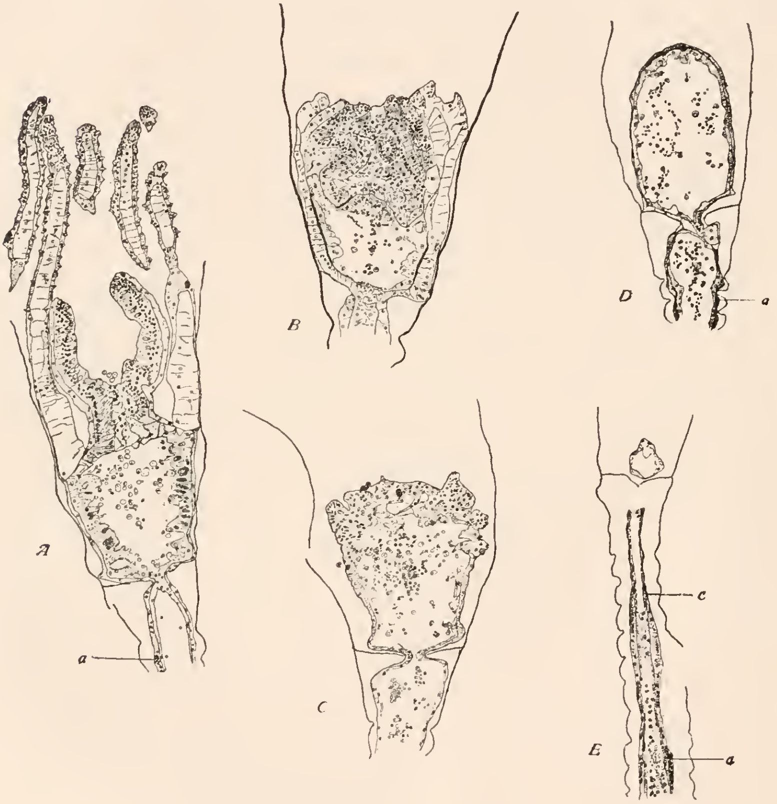
[1] "Recherches expérimentale sur la Régénération et l'Hétéromorphose chez astroides calycularis et Pennaria Carolinii," *Archives de Biologie*, XIX., 1902.

These results show that contact cannot in any case be considered the only factor to which the absorption of campanularian polyps is due, and that the process closely resembles that in other polyps in which under similar conditions we find either absorption or direct shedding of the hydranths with subsequent regeneration.

The material for study was obtained fresh each day, so that the animals should be in thoroughly good condition. Pieces of *Campanularia* were then cut and laid in watch crystals in contact with the glass in the way described by Loeb. The stages in the absorption of *Eudendrium* and *Pennaria*, which I used for comparison, being more difficult to obtain, were taken whether in contact or not, according to where they presented themselves. All the material was killed in cold corrosive acetic, and stained with Delafield's hæmatoxylin and congo red.

Within a few minutes after the removal of a piece or stalk, the cut end closes over, and the digestive current begins to flow slowly from one end of the hydroid to the other. It passes forward, and then is driven backward mainly by the contraction of the circular muscles of the polyps in the region just below the tentacles, but not involving a contraction of the whole animal; a slight pause occurs between each change in direction. The irregularity in the contraction of the polyps sometimes complicates the course of the current. At first the polyps remain expanded, and the only change noticeable is in the digestive fluid which becomes more and more laden with spherical granules of all sizes. The current is sometimes driven with such force that the contents break their way through a newly formed stolon or through the mouth of the polyp. The animal has up to this time been fully expanded except for the rhythmic contractions which decrease only the diameter of the body, but now it gradually contracts into its cup, and the body becomes shorter and broader, the latter change being largely due to the thickening of the ectoderm as can be seen even in the living animals. The tentacles undergo excessive contraction, becoming a crown of mere stubs, and then disappear altogether; their cells passing into the cavity of the polyp. At the same time, the hypostome absorbs.

These changes take some time and normally occupy at least two thirds of the time required for the complete disappearance of the polyp; sometimes the digestive current may, at this stage, distend the degenerating polyps and delay absorption for several hours. The usual time required is from six to twelve hours, but under the same conditions it may last from one to two days. The size of the structure left in the cup becomes slowly less and less, and at last the tiny ball of matter is drawn into the stem. I examined the living material carefully for signs of the breaking of the protoplasmic threads that stretch from the cœnosarc to the perisarc just below the cup, but I was unable in most cases to find any trace of it, until the last stage. At that time the strands break and the cœnosarc is drawn out in a fine thread. The protoplasm has been under a strain for the greater part of



the time, due to the growth of the stolon, but the protoplasm of the polyp cannot apparently be *drawn* through into the stem until it has reached a certain stage in its absorption.

The finer structure of normal *Campanularia* is as follows: The ectoderm cells which are flat on the body become cubical on the hypostome; there are no nettle cells except an occasional wandering one, until we come to the upper half of the tentacles. Below the cup lie masses of nettle-forming cells, somewhat irregular in their position, but never found in an quantity anterior to the first annulation. The endoderm is well differentiated on the hypostome into deeply-staining goblet cells and long spindle-shaped cells; in the walls of the body cavity there are large, clear endoderm cells and smaller granular gland cells. The tentacles contain a single row of endoderm cells. These are separated from those of the body cavity by a lamella at the base of the tentacle. Signs of change first arise in the endoderm of the body and the digestive current becomes filled with degenerating endoderm and gland cells, pinched-off portions of cytoplasm and loose nuclei. This process continues for some time without the appearance of any other change, except that as the endoderm becomes less, the lamella slowly contracts, becoming correspondingly thicker, and the ectoderm, having less surface to cover, changes from a thin layer to a much thicker one. The tentacles have also contracted to an abnormal extent, and at last by the breaking of the lamella across their base the endoderm cells round up and pass out into the body cavity. At this stage the tentacles are crowded together, and, the ectoderm being thrown into folds by the excessive contraction, frequently give, in surface view, the effect of being fused, as stated by Loeb. But by careful study the independence of the tentacles can be traced in spite of the closeness with which they are pressed together.

Soon after the endoderm has begun to pass out from the tentacles the lamella breaks near the tip and masses of nettle and ectoderm cells are poured into the cavity. The hypostome also degenerates, the ectoderm cells passing out rapidly into the digestive current and the lamella contracting after them. Soon the lamella of the hypostome breaks and disappears and the mass of ectoderm is also turned in. The polyp is now simply a shell of ectoderm and endoderm which are separated by the elastic lamella, which usually meets more or less completely at the oral end after the material of the tentacles and hypostome has been absorbed. At this time the lamella breaks in places and more cells from the ectoderm pass through. There is also a small amount of degeneration on the outside, and by these means the amount of ectoderm rapidly diminishes. Gradually the structure becomes smaller and smaller and finally the last fragment is drawn out of the cup. If there are many cells loose in the body cavity of the polyp at this time, they frequently break through the thin wall and pass out into the water.

The best guide by which to determine the amount of protoplasm drawn into the stem, was found to be the masses of nettle-forming cells before alluded to. The cells really drawn represent a very small fraction of the original number. The greater majority have been thrown into the digestive current, from which many are absorbed by the endoderm cells throughout the entire colony.

To compare the process in *Campanularia* with that in other hydroids, I examined both *Eudendrium* and *Pennaria* in which "absorption" also occurs and found the process again one of degeneration. From the time when the first degenerating masses are seen in the digestive current to the final drawing through of the small degenerated mass, the method is almost identical with that in *Campanularia*.

Recently there has appeared a paper by Gast and Godlewski, Jr., on the degeneration of the polyps of *Pennaria*[1] who have obtained results similar to my own.[2] It is interesting to note that their material was taken from polyps which had regenerated their heads in the laboratory, and then after two or three days had begun to absorb again — a different condition from that under which mine were obtained, yet the process is the same. Since these investigators have fully covered the ground for *Pennaria*[3] I shall not describe the changes in that form and indeed merely speak of two or three points in the degeneration of *Eudendrium* that differ from that in *Campanularia*. The degeneration of the endoderm is much more rapid, the cells breaking down more completely and filling the digestive cavity with fine protoplasmic granules. Since there is no lamella across the bases of the tentacles, the endoderm can also pass out from them more readily. The loss of ectoderm is here also accomplished by the passing in of cells through breaks in the lamella, the edges of which are apt to draw together again. The complete disappearance of the lamella does not occur until a very late stage. At the end the whole of the remaining structure is not always drawn through into the stalk, but an ill-defined mass of protoplasm is often left at the end.

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[1] "Ueber den Regulationsersheinungen bei Pennaria carolinii," *Archiv für Entwickelungsmechanick der Organismus*, XVI., 1903.

[2] See preliminary note, BIOL. BULL., IV., 2, 1903.

[3] Probably another species.

The constant position of the ectodermal gland cells near the beginning of the stalk throughout the degenerative changes show that there is no drawing of cells into the stem until the final stages.

The histological evidence thus supports my observations on the living animals, that in *Campanularia* we have to do with no transformation of the protoplasm due to contact, but with a degeneration of the polyp. Similar changes take place in other hydroids, and occur apparently when they are subjected to abnormal or harmful conditions.

I wish to express my thanks to Professor Morgan for his suggestions and kind supervision of my work.

FORM REGULATION IN CERIANTHUS.

II. The Effect of Position, Size and Other Factors upon Regeneration.

C. M. CHILD.

In the preceding paper (Biol. Bull., Vol. V., No. 5, 1903), the course of regeneration in cylindrical pieces from the middle region of the body was described, since such pieces afford a typical result and serve as a basis for comparative study. It is desired in the present paper to call attention to certain conditions which influence the result, either as regards time or quantity.

The principal features in the regeneration of *Cerianthus* may be reviewed as follows: the collapse of the piece after section and the infolding of the ends; the closure of the ends by new tissue and the gradual distension of the piece and the increase in the area of the new tissue at the ends in consequence of the accumulation of water in the enteron, probably by diffusion through the body-wall; the reduction and disappearance of the muscular layer and pigment at both ends; the regeneration of mesenteries; the outgrowth from the tentacular ridge of a marginal tentacle over each intermesenterial chamber; the formation of the mouth in the directive radius; the appearance of the labial tentacles in a circle upon the disc; the outgrowth of new tissue at the aboral end of the piece.

Since each of these processes is gradual it is impossible to determine with exactness the time of its beginning; moreover, the various processes overlap and are connected in such a manner that it is difficult to separate distinct stages except arbitrarily. For these reasons the comparison of different pieces with a view to determining the conditions which effect regeneration can best be accomplished by the examination of these pieces at stated times, rather than by noting the time at which a given piece arrives at a particular stage. The former method not only allows direct comparison of the pieces, and thus often renders the detection of slight differences less difficult, but it obviates the necessity for almost continuous observation and the accompanying manipulation necessary to examination, which is a source of irritation to the regenerating pieces and may often effect the result by causing rupture of new tissue or other injuries.

In general then the method pursued in the experiments was that of examining at intervals pieces to be compared and noting the condition of each. Owing to the number of points to be observed and the necessity for indicating slight differences any arrangement of the results in tables is unsatisfactory: they are given, therefore, in much the same manner as first recorded. The series of experiments described are selected from a large number but the results were remarkably uniform in all cases. In the description of the stages only the most salient features of the regeneration are mentioned in most instances. In all cases, however, unless definite statement is made to the contrary, regeneration proceeded in the typical manner.

I. DESCRIPTION OF EXPERIMENTS.

SERIES 22.[1]

September 24, 1902. The oral end, including the œsophageal region, was removed from twenty-three large specimens of *C. solitarius* and the remaining portion of the body was divided by a transverse cut as nearly as possible into two equal pieces (Fig. 1), oral halves being designated A, aboral B. All of the pieces A were placed in one aquarium, all of B in another.

September 27: Three days after section:

A. Most of the pieces are still collapsed, but in a few the ends are closed and a slight distension with water is evident.

B. All still collapsed.

September 28: Four days after section:

A. All the pieces are more or less distended with water: three pieces show the tentacular ridge and the first traces of marginal tentacles.

B. Three pieces are closed and somewhat distended, the remainder still collapsed.

September 29: Five days after section:

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[1] The series retain the numbers given them in my notes.

A. Distinct marginal tentacles are present on eight pieces; the remainder all distended and with tentacular ridge.

B. All closed and more or less distended; in a few distension is just beginning; none with distinct tentacles.

September 30: Six days after section:

A. All with distinct marginal tentacles from 0.5–1.0 mm. long.

B. The pieces which were the first to close and become distended show traces of marginal tentacle buds; all pieces distended with water.

October 1: Seven days after section:

A. All with marginal tentacles 1.0–2.0 mm. long.

B. Traces of marginal tentacles on all pieces except those which were the last to close.

This series was not kept under observation for the later stages. As regards the earlier stages, however, it shows clearly that the aboral pieces regenerate somewhat less rapidly than the oral pieces, although the latter are cut at both ends, the former at only one end. The difference between the two sets of pieces is universal, not even the most advanced pieces in the set B showing as rapid regeneration as the least advanced of A. In general the differences between pieces of the same set are slight.

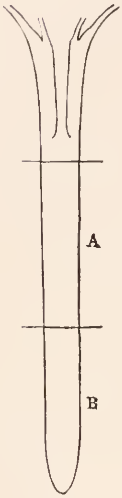

Fig. 1.

Series 45.

November 7, 1902. Tentacles and disc were removed from four specimens and the remaining portion of the body was cut into four pieces, A, B, C, D, as nearly equal as possible (Fig. 2). All of the pieces A were placed in one aquarium, all of B in another, etc. The pieces A contained a part of the œsophagus.

November 9: Two days after section:

A. Ends closed and piece distended; as in other similar cases the cut oral margin of the œsophagus has united with the oral margin of the body-wall so that the pieces possess a well-developed mouth-opening.

B, C, D. All collapsed.

November 10: Three days after section:

A. Marginal tentacular ridge appearing.

B. One piece beginning to fill with water; others collapsed.

C. One piece beginning to fill; others collapsed.

D. All collapsed.

November 12: Five days after section:

A. Marginal tentacular ridge distinct, with fading pigment.

B. All distended; new tissue at ends visible; tentacular ridge forming.

C. One piece distended; new tissue at ends visible; two pieces partly filled but not sufficiently to show the new tissue at the ends; one piece still collapsed.

D. All still collapsed.

November 15: Eight days after section:

A. Marginal tentacles just appearing in all.

B. One piece with marginal tentacles just appearing; three pieces distended; new tissue at ends visible; tentacular ridge distinct, unpigmented.

C. Two pieces distended; new tissue at ends visible; tentacular ridge forming; one piece still collapsed; one piece collapsed and completely enclosed in slime which was removed.[1]

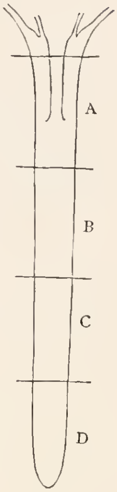

FIG. 2.

D. All still collapsed.

November 20: Thirteen days after section:

A. Marginal tentacles in all 1 mm. in length.

B. In one piece marginal tentacles 1 mm., in others about 0.5 mm.

C. All distended; tentacular ridge unpigmented, marginal tentacles just appearing.

D. One piece beginning to fill, others collapsed.

November 25: Eighteen days after section:

A. Marginal tentacles 2–2.5 mm. showing faint traces of pigmented bands in two pieces, in other two unpigmented; labial tentacles 0.5 mm. At aboral end new outgrowth 2 mm.

B. Marginal tentacles 1–2 mm., some differences in length appearing in individual pieces, unpigmented; a few labial tentacles just appearing in each piece. At aboral end new outgrowth about 2 mm.

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[1] The complete enclosure of pieces in slime, as in a cyst, often occurs when they remain collapsed for more than four or five days. The slime being secreted all over the ectoderm unites at the infolded ends and forms a complete cyst from which the piece is unable to emerge after a few days.

C. In two pieces marginal tentacles 0.5–1.0 mm., in other two slightly less advanced; in all unpigmented: none with labial tentacles. At aboral end no distinct outgrowth of new tissue.

D. One piece partly filled, others collapsed.

December 2: Twenty-five days after section:

A. Marginal tentacles about 5 mm., with distinct transverse pigment bands; labial tentacles 1–1.5 mm. Aboral end as before.

B. Marginal tentacles 3.5–4 mm., pigment bands visible but lighter than in A; labial tentacles 0.5–1 mm. Aboral end as before.

C. Marginal tentacles mostly 3 mm., a few in two pieces 4 mm., all unpigmented; labial tentacles just appearing. At aboral end no distinct outgrowth of new tissue.

D. One piece partly filled, but enclosed in slime which was removed; others still collapsed.

December 12: Thirty-five days after section:

A. Marginal tentacles 6–7 mm., pigment bands dark: labial tentacles about 2 mm. At aboral end outgrowth of new tissue 2–3 mm.

B. Marginal tentacles 5–6 mm.; pigment bands lighter than in A; labial tentacles 1–1.5 mm. At aboral end outgrowth of new tissue 2–3 mm.

C. Marginal tentacles 4–5 mm.; pigment bands visible, but slightly lighter than in B; labial tentacles about 1 mm. At aboral end outgrowth of new tissue 1 mm.

D. One piece closed and partly filled as before, but no traces of tentacular ridge. Three pieces still collapsed and enclosed in slime which was removed.

The series as a whole was concluded at this time, since the only further changes in A, B and C consist of a slight increase in length of the tentacles and the pigmentation. The pieces D, however, which had not as yet shown any traces of regenerating tentacles were kept under observation until January 21, 1903. Up to this time only the one piece which had become partly filled showed any signs of regeneration, the others remaining completely collapsed and surrounded by slime, which was removed from time to time in order to permit distension to occur if there were any tendency. The changes during this time in the one piece which was closed and partly filled are of considerable interest. At one side of the closed oral end of the piece a few minute outgrowths 0.2–0.5 mm. in length made their appearance. They resembled marginal tentacles and were situated where these organs should appear, but there were only a few of them close together on one side and no others appeared. Fig. 3 shows the piece as it appeared January 21. The new tissue closing the end is indicated by the stippling. At one side are six small outgrowths resembling tentacles, but no traces of any others can be found at any point of the circumference. At the conclusion of the experiment the piece was opened and it was found that a few of the longest mesenteries extended into the piece in the radius in which the outgrowths appeared. This region is then without doubt the region of the directive mesenteries, and the mesenteries present are simply the longest mesenteries of the body which lie to the right and left of the short directives and extend nearly to the aboral end. The small outgrowths correspond in position with the spaces between these mesenteries and there can be little doubt that they represent marginal tentacles. No other mesenteries are present in the piece, none having regenerated. It becomes evident from the history of this piece that the presence of mesenteries is necessary for the regeneration of marginal tentacles. In pieces from regions nearer the oral end mesenteries are regenerated, but in this piece no trace of regenerated mesenteries could be found, and tentacles have begun to regenerate only in the spaces between such of the old mesenteries as extended into the piece.

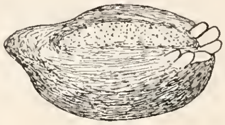

Fig. 3.

The series as a whole affords several results of importance. As in the preceding series, the decreasing rapidity of regeneration with increasing distance from the oral end of the body is clearly shown. The pieces A regenerate more rapidly than B, B more rapidly than C, and finally D, the aboral pieces, are capable of only a slight degree of regeneration or of none at all, the difference between the one piece which regenerated a few tentacles and the others probably being due to the fact that the cut separating C from D in the one case was slightly more oral than in the other three.

The differences in the rapidity and the amount of regeneration are best shown at the oral ends of regenerating pieces, for it is difficult to determine with exactness the amount of actual new tissue at the aboral ends of the pieces, since the line of demarcation between the unpigmented tip and the normally pigmented regions oral to it is not at all sharp, extending in many cases over two to three millimeters. As regards the aboral ends the pieces A and B showed little difference, but regeneration at the aboral end of C was in all cases distinctly less than in A and B.

In general the series seems to indicate that not only is regeneration less rapid with increasing distance from the oral end, but that there is a corresponding difference in the amount of regeneration. In the pieces A, B and C the differences are comparatively slight, though without doubt present as can be seen by comparing the data for these pieces thirty-five days after section. When the pieces D are taken into consideration, however, the difference between these and all other pieces is marked, for in no case did these aboral ends show anything approaching complete regeneration. There is then, according to these results, a rapid decrease in regenerative power near the aboral end of the body, and apparently complete absence of this power in an aboral region representing approximately one fifth of the body-length. As will be shown below, much smaller pieces than this from other regions of the body are capable of complete regeneration; moreover, the size of the area within which regeneration does not occur differs according to conditions.

SERIES 54 AND 55.

December 15, 1902. The tentacles, disc and œsophageal region were removed from twenty large specimens; ten of the remaining pieces were then divided by a transverse cut into two pieces, A and B (Fig. 4), the cut being made near the aboral end so that the pieces A comprised the greater part of the body aboral to the œsophagus, while the pieces B represented the extreme aboral end, about one sixth of the body-length. These two sets of pieces constituted Series 54.

Each of the remaining ten pieces was also divided by a transverse cut into two pieces A and B, but in this case the cut was made near the oral end of the piece (Fig. 5), so that A represents one sixth of the body-length from the region just aboral to the œsophagus, while B is the whole remaining portion of the body. These pieces constitute Series 55.

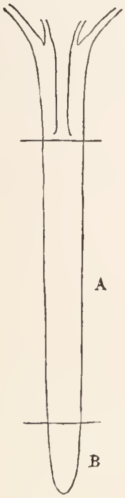

FIG. 4.

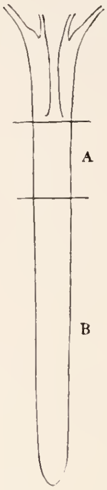

FIG. 5.

In this manner four sets of ten pieces each were obtained. The oral ends of pieces 54A and 55A represent approximately the same level in the body of the individuals from which they were taken, while their aboral ends lie at very different levels; moreover, the pieces 54A are about four times as long as 55A. The oral ends of the pieces 54B and 55B are at very different levels, while their aboral ends are the aboral ends of the parent bodies; 55B is about four times as long as 54B.

In 54A and 54B we have pieces differing widely in size and with oral ends at very different levels; the same is true of 55A and 55B, but the relations of size are reversed.

The comparative study of the regeneration of those four sets of pieces should afford data regarding the rapidity and amount of regeneration at different levels of the body and in pieces of different sizes.

December 19: Four days after section:

54A. All with closed ends and filling with water, but ends not yet expanded so as to show new tissue.

54B. All collapsed.

55A. All with closed ends and filling with water, but ends not yet expanded. Condition same as in 54A.

55B. Ends closed, but pieces contain less water than 54A and 55A.

December 22 : Seven days after section :

54A. All distended, ends expanding, new tissue visible ; tentacular ridge just appearing.

54B. All collapsed.

55A. Similar to 54A.

53B. All filling with water, but none distended sufficiently to expand the ends and show new tissue.

December 24 : Nine days after section :

54A. Tentacular ridge distinct, with fading pigment.

54B. All still collapsed.

55A. Similar to 54A.

55B. All distended, ends expanded and tentacular ridge just appearing.

December 26 : Eleven days after section :

54A. Marginal tentacles just appearing in some specimens as minute outgrowths from tentacular ridge, which is now unpigmented.

54B. All still collapsed.

55A. Similar to 54A.

55B. Tentacular ridge with fading pigment, but less distinct than in 54A ; no marginal tentacles.

December 28 : Thirteen days after section :

54A. Marginal tentacles 0.25–0.5 mm.

54B. All still collapsed.

55A. Similar to 54A.

55B. Tentacular ridge distinct, unpigmented in most cases ; in a few the earliest traces of marginal tentacles visible.

January 3, 1903 : Nineteen days after section :

54A. Marginal tentacles 2–3 mm.

54B. All still collapsed, much contracted, rounded in form.

55A. Similar to 54A.

55B. Marginal tentacles 1–1.5 mm.; in one specimen with rather unequal tentacles a few 3 mm.

January 11: Twenty-seven days after section:

54A. Marginal tentacles 5–6 mm.; transverse bands of pigment distinct; labial tentacles 1–1.5 mm. At aboral ends no well-marked outgrowth of new tissue; ends slightly lighter in color at region of closure.

54B. All still collapsed, much contracted, rounded in form.

55A. Oral ends similar to 54A. At aboral ends a distinct outgrowth of new tissue 2–3 mm.

55 B. Marginal tentacles 3–5 mm.; pigmentation of tentacles slightly less deep than in 54 A; labial tentacles just visible — 1 mm.

January 21: thirty-seven days after section:

54 A. Marginal tentacles 7–8 mm.; labial tentacles 1–1.5 mm. No distinct outgrowth of new tissue at aboral ends.

54 B. Collapsed, rounded and still further reduced in size.

55 A. Marginal tentacles 6–8 mm.; labial tentacles 1–1.5 mm. At aboral ends distinct outgrowth of new tissue 3–5 mm. At this time the average length of the marginal tentacles in these pieces is somewhat less than in 54 A. In the latter cases there are fully as many specimens with tentacles 8 mm. in length as with tentacles 7 mm. In 55 A, however, only a few pieces, and these the largest, possess marginal tentacles 8 mm. in length; in nearly all the marginal tentacles are 6–7 mm. Moreover, the average length of the labial tentacles in 55 A is slightly less than in 54 A. These pieces are evidently falling behind the longer pieces.

55 B. Marginal tentacles 5–6 mm., somewhat less deeply pigmented than in 54 A; labial tentacles 1 mm.

At this time the regenerated structures had acquired their maximum size; afterward reduction in size, which always occurs in the pieces kept without food, began. For present purposes it is not necessary to follow the history of these pieces further.

Comparison of the data afforded brings to light a number of interesting results. Comparing the rapidity of regeneration in the different pieces, it is seen that the oral ends of pieces 54 A and 55 A, which represent approximately corresponding regions of the parent body, regenerate with equal rapidity except at the end of the experiment, although pieces 54 A are about four times as long as pieces 55 A. The oral ends of pieces 55 B, which represent a region of the parent body further aboral than those of 54 A and 55 A, regenerate less rapidly than these, although the pieces are about equal in size to 54 A and four times as long as 55 A. And finally, the pieces 54 B, whose oral ends represent a region near the aboral end of the parent, do not regenerate at all.

As regards the aboral ends of the pieces only 54A and 55A need be considered, since no regeneration occurs at the aboral end of a piece when this represents the aboral end of the parent-body, as is the case in 54B and 55B. In 54A and 55A the difference in the rapidity and amount of regeneration at the aboral ends is marked; in 55A, where the aboral ends of the pieces represent a region oral to the middle region of the parent-body the aboral regeneration was much greater than in 54A, where the aboral ends represent a region near the aboral end of the parent-body, even though the pieces 54A were four times as long as 55A.

From all of these facts it is evident that the rapidity and amount of regeneration decrease as the cut surface, either oral or aboral, approaches the aboral end of the parent-body, and that the size of the piece has no marked influence, at least within the limits of size of the present experiment. That the size of the piece does, however, affect the final result in some degree is shown by the condition of pieces 54A and 55A at the end of the experiment 37 days after section; while no differences between the two sets were noted earlier it was found at this time that the smaller pieces 55A were falling slightly behind the larger 54A. Here then a slight influence of size is noticeable, though only in the later stages of the experiment. As will be shown later this result is confirmed by other cases. In pieces above a certain minimal size regeneration is not influenced by the size, except in the later stages.

SERIES 35.

October 20, 1902. In this case after removal of disc and tentacles a single specimen was cut into four pieces, A, B, C, D as shown in Fig. 6. The piece B was much smaller than the others and masses of the mesenterial filaments protruded from each end, thus delaying the closure and normal regeneration; it is therefore omitted from the present consideration. The pieces A and C are nearly equal in length and are about two thirds the length of D.

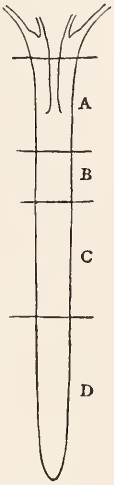

FIG. 6.

October 22: two days after section: All pieces still collapsed.

October 23: three days after section:

A. Margin of œsophagus united with body-wall, aboral end closed and enteron partly filled with water.

C. and D. Both still collapsed.

October 24: Four days after section:

A. Sufficiently distended with water to spread the inrolled margins and allow the œsophagus at the oral end and the new tissue closing the aboral end to become visible.

C. and D. Both still collapsed.

October 25: Five days after section:

A. Distended with water: tentacular ridge visible and pigment disappearing from it.

C. Ends closed by new tissue; distended.

D. Enteron partially filled with water; distension not yet sufficient to separate the infolded oral margins and permit new tissue to become visible.

October 27: Seven days after section:

A. Marginal tentacles just appearing on tentacular ridge.

C. Tentacular ridge distinct; its pigment disappearing.

D. Distended with water; new tissue closing oral end exposed by separation of cut margins in consequence of distension: tentacular ridge visible, with fading pigment.

October 29: Nine days after section:

A. Marginal tentacles 1 mm.

C. Marginal tentacles just appearing on tentacular ridge.

D. Tentacular ridge distinct, without pigment; no tentacles visible.

October 31: Eleven days after section:

A. Marginal tentacles 2 mm. At aboral end new tissue growing out in a small point 1.5 mm.

November 6: Seventeen days after section:

A. Marginal tentacles 5 mm.; labial tentacles 0.5–1 mm. At aboral end outgrowth of new tissue 2 mm.

C. Marginal tentacles 3–4 mm.; labial tentacles 0.5. At aboral end of outgrowth of new tissue 1 mm.

D. Marginal tentacles 1–2 mm.; labial tentacles not yet visible.

November 12: Twenty-three days after section:

A. Marginal tentacles 10 mm.; distinctly marked with the characteristic transverse bands; labial tentacles 2–3 mm. At aboral end margins of old body-wall are becoming involved in the growth and losing pigment; the unpigmented area, including outgrowths, is about 3 mm. in length from aboral end.

C. Marginal tentacles 6–7 mm.; transverse pigment bands visible but not dark as in A; labial tentacles 2 mm. At aboral end new outgrowth 1.5 mm.

D. Marginal tentacles 5–6 mm. still unpigmented; labial tentacles 1 mm.

November 20: Thirty-one days after section:

A. Marginal tentacles 12–13 mm.; pigment bands dark and distinct; labial tentacles about 3 mm. Aboral end as before.

C. Marginal tentacles 8–9 mm.; pigment bands distinct but less dark than in A; labial tentacles 2–2.5 mm. At aboral end unpigmented area 2–3 mm. in length.

D. Marginal tentacles 6–8 mm.; pigment bands visible but less dark than in C; labial tentacles 2 mm.

December 2: Forty-three days after section:

A. Marginal tentacles 12–13 mm.; labial tentacles 3–4 mm. At aboral end the new outgrowth is becoming pigmented.

C. Marginal tentacles 10 mm.; pigmentation of tentacles scarcely distinguishable from that of A; labial tentacles 3 mm. At aboral end the unpigmented area about 3 mm.

D. Marginal tentacles 12–13 mm.; pigmentation slightly less dark than that of A; labial tentacles 3–4 mm.

At this time regeneration is essentially complete in the pieces; no further increase in the length of tentacles, or of the new growth at the aboral end occurs. The marginal tentacles of C and D are still slightly lighter in color than those of A, and the pigment has not yet extended over the aboral outgrowth in C as far as in A, but these slight differences are later eliminated.

Examination of the data shows that at all stages except the final A is more advanced in regeneration than C, and C more advanced than D.

It will be noted also that the regenerated parts of piece A did not increase in size after 31 days, with the exception of the labial tentacles which showed a slight increase between 31 and 43 days. In the piece C a slight increase in the length of all tentacles occurred between 31 and 43 days. In the piece D, however, there was a marked growth during this time. In other words the piece A completed its regeneration first, then the piece C, and last of all the piece D.

Throughout this series then there is a distinct relation between the rapidity of regeneration and the position of the pieces in the parent-body, the rapidity of regeneration decreasing with increasing distance from the oral end.

One other point requires consideration : the regenerated tentacles of the piece D finally attain the same length as those of piece A. This would appear at first glance to contradict the results obtained from other series of experiments where not only the rapidity but the amount of regeneration diminishes toward the aboral end. Comparing A and C, two pieces about equal in size, we find that the amount of oral regeneration in A is greater than in C, as might be expected from comparison with other series, since C represents a region farther from the oral end of the parent-body than A. The piece D, still nearer the aboral end of the parent-body, but much longer than A and C, while regenerating more slowly than either of these finally equals A in the amount of regeneration. Apparently in this case the influence of size has counterbalanced the influence of position. If piece D was of the same size as A and C the amount of oral regeneration would undoubtedly be less than in those pieces, but since it is much larger, *i. e.*, contains much more available material, regeneration continues for a somewhat longer time (note the increase in size of tentacles in D between 31 and 43 days) and the regenerated organs finally, though after a longer time, reach a condition similar to that in A. In this case, as in Series 54 and 55, the influence of size is slight and appears only in the latest stages of regeneration.

Series 56.

December 15, 1902. Disc, tentacles and œsophageal region were removed from ten large specimens by a transverse cut aboral to end of œsophagus. The aboral piece was then cut into two pieces, A and B, of equal length (Fig. 7) which were kept for comparison.

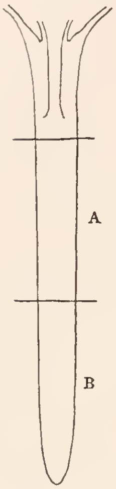

Fig. 7.

December 19: four days after section:

A. Nine pieces with ends closed; a few distended, the others partly filled with water; one piece still collapsed.

B. All still collapsed.

December 22; seven days after section:

A. All distended; ends well expanded, showing new tissues in a few pieces the tentacular ridge is just appearing.

B. All still collapsed.

December 26: Eleven days after section:

A. Tentacular ridge distinct, with faded pigment; in a few pieces the first traces of marginal tentacles distinct.

B. Filling with water but not distended; new tissue at oral end not visible.

December 28: Three days after section:

A. Marginal tentacles 0.5 mm.

B. Four pieces fairly well filled with water; tentacular ridge just visible; six pieces collapsed or only partly filled; tentacular ridge not visible.

January 3, 1903: Seventeen days after section:

A. Marginal tentacles 2–3 mm.

B. One piece distended; marginal tentacles 2 mm. Nine pieces partly or completely collapsed; no tentacular ridge or tentacles visible.

Circumstances necessitated the conclusion of the series at this time, so that it was impossible to determine whether the nine pieces of B would ever have regenerated. The series affords, however, some interesting results. As in all other series regeneration is much less rapid in the aboral pieces; in only one case did the aboral pieces regenerate tentacles before the conclusion of the experiment. Examination of the data shows that the pieces B were all filling with water eleven days after section ; that two days later all but four were collapsed, and that, finally, seventeen days after section, only one piece was filled with water. These changes are undoubtedly due to the fact that the growth of new tissue at the ends of these pieces failed to keep pace with the pressure of water in the enteron, and so rupture occurred as soon as the pieces reached a certain point. In only one case did the new tissue remain intact, viz., the case in which tentacles appeared.

As is evident from a comparison with other series, viz., series 22, 35, and 45, regeneration was found to occur in other cases in pieces representing about the aboral third of the body, though pieces representing the aboral fifth (series 45) or less did not regenerate. Why then did regeneration fail to occur in the aboral pieces of the present series? The difference is undoubtedly to be accounted for by the low temperature of the water. This series was begun in December and continued into January. The temperature of the water was very much lower at this time than during the autumn, and several other series begun on the same date showed similar results. In other words, the area at the aboral end which is incapable of regeneration increases as the temperature becomes lower, and in the present series includes more than the aboral third of the body. This point will be discussed in a following section where the influence of temperature is considered.

(*To be Continued.*)

ARTIFICIAL MIXED NESTS OF ANTS.

ADELE M. FIELDE.

Mixed nests of ants are rarely found in nature, and the ants associated in such nests are always of the same subfamily if not of the same genus.[1]

There are two ways of causing ants of different genera, or even of different subfamilies, to live peacefully together. One way is that of destroying the sense of smell in the ants by depriving them of a portion of the antennæ. Forel discovered, in the seventies, that the funicles were the organs of smell. I have had representatives of three subfamilies of ants, all without funicles, living amicably together through several consecutive weeks, although the members of the group varied in size, from the huge *Camponotus pennsylvanicus* to the small *Stenamma fulvum;* in form, from the shark-like *Stigmatomma pallipes* to the chubby *Lasius umbratus;* in color, from the jet-black *Cremastogaster lineolata* to the amber-yellow *Lasius latipes;* and in character, from the truculent *Myrmica rubra* to the patient *Formica subsericea.*

In 1901, using *Stenamma fulvum* for the experiments, I located[2] the appreciation of the nest-aura in the distal segment of the funicle, the eleventh; that of the colony, in the tenth segment; that of the individual track, in the ninth segment; that of the inert young, in the eighth and seventh segments. I have lately located the appreciation of the odor of enemies in the sixth and fifth segments.

I cut off the five distal segments of the antennæ from seven queens[3] of *Stenamma fulvum*, seven queens of *Cremastogaster lineolata*, five queens of *Myrmica rubra*, five queens of *Lasius*

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[1] E. Wasmann, "Die zusammengesetzten Nester und gemischten Kolonien der Ameisen," 1891. William Morton Wheeler, "The Compound and Mixed Nests of American Ants," *American Naturalist*, 1901.

[2] A. M. Fielde, "Further Study of an Ant," *Proceedings of the Academy of Natural Sciences of Philadelphia*, November, 1901.

[3] Among the Myrmicine ants, queens only were used for these experiments, because of the abnormal irritability of myrmicine workers lacking parts of the antennæ.

umbratus, seven workers of *Lasius latipes*, five workers of *Camponotus pennsylvanicus*, four workers of *Formica sanguinea*, four workers of *Formica subsericea*, and three workers of *Stigmatomma pallipes*, and when these ants had recovered from shock-effect, with healed wounds, I placed them all in an artificial nest, roomy for their number, having thirty-two square inches of floor-space. Duels were constant, and in two hours there were but twenty-three survivors from the forty-seven ants. Several of the survivors were disabled.

I then formed a new group, with other ants, having the four proximal segments of the funicle intact. This group included representatives of the Camponotines, *Camponotus pennsylvanicus* and *Formica sanguinea;* of the Myrmicines, *Stenamma fulvum* and *Cremastogaster lineolata*, and of the Ponerines, *Stigmatomma pallipes.* These lived peacefully together many days, in one of my small Petri cells, and ants of different subfamilies often huddled together. In this cell I saw a queen of the *Stenammas* lapping regurgitated food from the mouth of a *Camponotus* worker.

In another mixed group, made up of ants retaining from three to six segments of a funicle, I removed and examined every ant that attacked one of another species, and found that all such ants retained more than four segments of the funicle.

We may, then, secure peaceful mixed nests by depriving the inmates of certain segments of the antennæ.

I have lately created many mixed nests by another method, that of educating the ants in ant-odors unlike their own.[1] If one or more individuals, of each species that is to be represented in the future mixed nest, be sequestered within twelve hours after hatching, and each ant so sequestered touch all the others with its antennæ during the three ensuing days, these ants will live amicably together thereafter, although they be of different colonies, varieties, species, genera or subfamilies. For sequestering the ants, I used artificial nests, made in watch-glasses so small that the natural movement of the newly-hatched ants would bring each of them into contact with all the others. In no case did the callows quarrel, and those of most diverse lineage sometimes snuggled one another. The ant's sense of smell appears to be perfectly acquired, and its standards of correct ant-odor to be established during the first three days after hatching. Any two species or any number of species that I captured for use in these experiments, became accustomed to each other's odor, and therefore friendly, if the early association was close and continuous. This association is more perfect when no inert young distracts the attention of the callows from one another, and when the arrangement of the nest offers no place of seclusion for any of its inmates. Air, humidity and nourishment were provided as in large nests of the Fielde pattern. When the ants had been thus segregated for five days or more, the inmates of several like nests were transferred to a more spacious habitation, and newly hatched ants from the same colonies could be safely added thereafter; but no ant of other lineage nor of greater age was amicably received in any of the mixed nests.

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[1] The experiments were made at the Marine Biological Laboratory at Woods Hole, Mass., in July, August and September, 1903.

Each of the groups mentioned in the following list existed under my care for a month or more after the cessation of additions of newly hatched ants to their mixed nest.

Myrmicine Ants.

Group 1.—Six queens of *Cremastogaster lineolata* with eighty workers of *Stenamma fulvum.* The workers snuggled the queens as closely as if of the same species as themselves. In each of two watch-glass nests, the sole queen died on the third day after hatching. Newly-hatched queens of the same *Cremastogaster* stock were accepted by the bereaved workers,[1] in the one case three days, and in the other case five days, after the death of their first queen. I know no adult ants that will accept a queen from another colony of their own species, much less a queen of a genus not their own.

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[1] After these ants in group 1 had been established for two days in a Fielde nest, a raid was made upon them by adult workers, of the queens' stock, that had escaped from the hatchery-nest, and hidden in a crevice in the laboratory. Very early one morning, I discovered that these adult *Cremastogaster* workers had entered the nest in considerable numbers through a rift in the towelling. Some of them were clustering around the young queens, while others were busily employed in dragging the *Stenamma* callows out of the nest. My arrival thwarted an apparent design of the *Cremastogasters* to eject the *Stenammas* and dwell in an unmixed nest with queens of their own.

This first group is noteworthy, because *Stenamma fulvum* and *Cremastogaster lineolata* will each feed their larvæ upon the eggs, larvæ and pupæ of the other. In one of my artificial nests the *Stenammas* lately took care, with their own young, of a great number of *Cremastogaster* larvæ and pupæ, during two months; but every *Cremastogaster* that hatched was instantly killed and cast upon the rubbish-heap.

Group 2. — *Myrmica rubra*, *Stenamma fulvum* and *Cremastogaster lineolata*, workers of each species.

ONE SPECIES OF CAMPONOTINE ANTS WITH ONE SPECIES OF MYRMICINE ANTS.

Group 3. — *Lasius latipes* with *Stenamma fulvum*; workers of each species.

Group 4. — *Lasius umbratus* with *Stenamma fulvum*; workers of each species.

Group 5. — *Lasius umbratus* with *Cremastogaster lineolata*; workers of each species.

Group 6. — *Formica sanguinea* with *Cremastogaster lineolata*; workers of each species.

Group 7. — *Formica subsericea* with *Cremastogaster lineolata*; workers of each species.

ONE SPECIES OF CAMPONOTINE ANTS WITH TWO SPECIES OF MYRMICINE ANTS.

Group 8. — *Formica sanguinea* with *Stenamma fulvum* and *Cremastogaster lineolata*; workers of each species.

TWO SPECIES OF CAMPONOTINE ANTS WITH TWO SPECIES OF MYRMICINE ANTS.

Group 9. — *Lasius latipes* and *Formica lasiodes*[1] with *Stenamma fulvum* and *Cremastogaster lineolata*; workers of each species.

Group 10. — *Camponotus pennsylvanicus* and *Formica sanguinea* with *Stenamma fulvum* and *Cremastogaster lineolata.*

ONE SPECIES OF CAMPONOTINE ANTS WITH THREE SPECIES OF MYRMICINE ANTS.

Group 11. — *Lasius latipes* with *Stenamma fulvum*, *Myrmica rubra* and *Cremastogaster lineolata*; workers of all four species with one queen of *Cremastogaster lineolata.*

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[1] Kindly identified for me by Dr. W. M. Wheeler.

THREE SPECIES OF CAMPONOTINE ANTS WITH ONE SPECIES OF MYRMICINE ANTS.

Group 12. — *Camponotus pennsylvanicus*, *Formica sanguinea* and *Formica subsericea* with *Stenamma fulvum;* workers of each species.

ONE SPECIES OF PONERINE ANTS WITH ONE SPECIES OF MYRMICINE ANTS.

Group 13. — *Stigmatomma pallipes* with *Stenamma fulvum;* queens of the former with workers of the latter.

ONE SPECIES OF PONERINE ANTS WITH ONE SPECIES OF CAMPONOTINE ANTS.

Group 14. — *Stigmatomma pallipes* with *Formica subsericea;* workers of each.

ONE SPECIES OF PONERINE ANTS, ONE SPECIES OF CAMPONOTINE ANTS AND ONE SPECIES OF MYRMICINE ANTS.

Group 15. — *Stigmatomma pallipes*, queens and workers, with workers of *Formica subsericea* and of *Stenamma fulvum.*

In my artificial mixed nests, there is a close affiliation of ants of different species. Those of different subfamilies sometimes lick one another. Introduced young is carried about and taken care of without regard to its origin. Ants of one genus accept regurgitated food from those of another genus.

Ants appear to associate readily with all harmless familiars. In the wild nests of *Stenamma fulvum* I often see gray sowbugs roaming about, and they do not molest the ants, nor are they molested by the ants. On my putting a sowbug into an artificial nest of these ants, they seemed to treat it sportively, two or three young ants sometimes mounting upon its back and riding there, like children making excursions on an elephant. In my artificial mixed nests, small ants often ride on large ones, or stand on their backs and lick their heads.

Natural mixed nests probably originate among ants that seek in their abodes the same degree of moisture and of warmth. The habitat of each species being determined by the food-supply, the humidity and the temperature, any two species finding the same habitat a congenial one, might form a mixed nest through an accidentally close association of their newly-hatched members.

Were the occupants of my artificial nests free to seek the habitation most agreeable to each species, they would doubtless soon separate. Perhaps they would never quarrel with each other on meeting; but they would certainly fight with all ants whose age and lineage were not the same as their own, or else the same as that of their quandam associates in the artificial mixed nest.

Marine Biological Laboratory,
Woods Hole, Mass., September, 1903.

A CAUSE OF FEUD BETWEEN ANTS OF THE SAME SPECIES LIVING IN DIFFERENT COMMUNITIES.

ADELE M. FIELDE.

If the blood of several ants of the same species be shed upon a morsel of sponge, the characteristic odor of the species is discernible upon the sponge, even by human nostrils. The odor may be pungent, acid, acrid, or musty, or may be like that of an animal or vegetable oil. Of the thirty-five hundred known species of ants, probably each has its distinctive odor.

Every ant recognizes its acquaintances through their odor and its own sense of smell. It is violently hostile to all ants bearing an unfamiliar scent, and is caressingly friendly with ants whose odor it has always known.

That ants of unlike species should be inimical one to another is less strange than the fact that those of the same species and variety, inhabiting the same localities, but living in different communities, should be as intensely antipathetic as are those of different species. With a view to ascertaining the cause of the animosity between such communities, I made in 1902, many experiments[1] with *Stenamma fulvum*, with results showing that the odor of the ants changes with their age, and that ants will not live amicably with those much older than any that inhabited the nest in which they were hatched.

If an ant be hatched in isolation, and the isolation be maintained until the ant has attained its adult strength and color, the odor of its own body is this ant's sole criterion of proper ant-odor, and it will affiliate with no ants other than those of the same lineage and of nearly the same age as itself. It will affiliate instantly with the queen-mother from whose egg it came and whose odor it inherits, and will identify and caress that mother though she be presented among five queens never before encountered. It will also affiliate with any of her progeny of the same age as itself, or with the progeny of her own sister of the same age.

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[1] A. M. Fielde, "Notes on an Ant," *Proceedings of the Academy of Sciences of Philadelphia*, December, 1902.

A difference of forty days in the ages of two ants produces a difference of odor appreciable by the ants. If many pupæ be taken from one colony, and the workers hatched therefrom on the same day be segregated; and then, later on, more pupæ be taken from the same colony and the workers hatched therefrom on the same day be likewise segregated and established in a nest with inert young, the younger group of ants will not permit the members of the older group to approach the young in their nest, provided always that there be forty days or more of difference in the age of the two groups. The degree of animosity exhibited is in direct ratio to the difference in the age.

An ant hatched in the first brood of a solitary queen associates during its earliest days only with its queen and with its sister-ants, all hatched in one summer. These workers know only ants that are less than a year old, and will never become acquainted in a friendly converse with ants older than themselves. As seasons pass, and more ants are annually hatched from the eggs of this queen or the queens among her offspring, the latest comers know the odors of those of their own year, and of each year gone by, up to that of the oldest in the common nest. One might say that the sense of smell in the ant is more highly cultivated if she live in an old community.

I have been personally acquainted for four years with the ants in a community, the C colony, whose domain is a hundred yards in its diameter. On August 22, 1901, I took queens, males and workers from the wild nest of this colony, and segregated a similar group in each of two Fielde nests, where I kept them two years. The queens were winged when captured, and were doubtless less than a month old. The workers were fully colored, and may have been a year or more older than the queens. No young was permitted to hatch in either nest, and there was no communication between the two nests nor with outside ants. On August 25, 1903, I united the two groups, then numbering four queens and twenty-five workers in one nest, and two queens and nineteen workers in the other nest. They all affiliated instantly with no sign of cognizance of their long separation. They had added years simultaneously and there was no difference of odor to occasion distrust among them.

I then introduced into the nest of the two united groups several very young ants taken that day from the wild nest. These callows were kindly received because the old ants all recognized an ant-odor with which they had formerly been acquainted, and this recognition was instant notwithstanding the fact that they had met no callows during two years. It is probable that an ant remembers during its lifetime any odor with which it has once been acquainted.

I then brought queens and workers from the same wild nest, housed them with their inert young in one of my artificial nests and left them to establish their nest-odor. A few days later I introduced into their nest marked queens and workers from the groups segregated two years previously. The marked queens were instantly accepted by the queens and workers in the latest nest. The marked workers were amicably received by all the queens, and by most of the workers in the latest nest, while a few nabbed them or dragged them away from the pupæ-pile. They were not killed but were denied by these few, the crowning mark of ant-esteem, permission to share in the care of the young. It thus appeared that ants as old as were these sequestered workers were not common in the summer of 1903 in the wild nest of the C colony, while queens two years old were known to all the ants taken from the wild nest.

Difference of food, drink and environment during two years had not caused a difference of ant-odor between the segregated ants and their ancient comrades.

The progeny of queens of unlike age but of the same community are unlike in odor.

Four queens of the C colony, captured by me before their swarming and while they were still winged, on August 22, 1901, were segregated with kings of their own colony in one of my nests which I here refer to as Section A. Two queens of the same colony hatched on August 5, 1902, from pupæ taken from the wild nest two days earlier. They mated with kings of their own colony on August 22, 1902, and were later on segregated with workers hatched in my artificial nests between August 8 and 28, 1902, from C colony pupæ. This nest I here refer to as Section B.

The ants in the two sections were fed with the same kinds of food on the same days and had in all respects similar environment.

On July 12, 1903, an ant-worker hatched from a pupa that had been previously removed from Section B, and isolated in a Petri cell. This worker was kept in isolation until she was six days old. I then introduced into her cell a worker, the offspring of a queen in section A, and she attacked this worker with great violence, although the worker was of an age precisely her own and had likewise been isolated from the pupa-stage. The only difference between the two lay in the age of their respective mothers, one queen mother being two years old and the other one year old. Neither of these callows had, previous to their meeting, ever smelled any other ant, and had they had the same odor they would have affiliated, as do similarly reared ants that are the progeny of the same queen or of sister queens.

On August 24, 1903, when the ant from Section A, used in the foregoing experiment, was forty-three days old and was occupied in the care of introduced larvæ, I put into her Petri-cell, where she had always lived alone, a callow five days old, reared in isolation from a pupa taken from Section B. The resident ant at once attacked and dragged the callow. In this case the offspring of the older queen attacked the offspring of the younger queen, though that offspring was much younger than herself.

Other experiments coincided in their results with the two here recorded.

A cause of feud between ants of the same species living in different communities is a difference of odor arising out of difference of age in the queens whose progeny constitutes the communities, and difference of age in the ants composing the community.

Marine Biological Laboratory, Woods Hole, Mass.,
September, 1903.

DIMORPHISM IN BLISSUS LEUCOPTERUS.

J. F. GARBER.

Two forms of the chinch bug are recognized by entomologists — the one having wings fully developed, the other having wings more or less abortive. Between the two extremes of fully winged and almost wingless all gradations exist. Where the short-winged form occurs it is usually intermixed with long-winged individuals. Such a mixture appears at certain times in abundance in the timothy meadows of northeastern Ohio. It was from Trumbull, Portage, Mahoning and Stark counties of this state that Professor F. M. Webster furnished the principal portion of the material for the present study.

The study was undertaken with the direction of Professor C. B. Davenport to determine by quantitative methods the biological significance of the dimorphism.

Method.

The insects examined represented several random collections from different points. For study they were taken from the various bottles with no attempt at selection so those studied are presumed to present fairly the conditions in the whole group.

Where practicable, the wings were carefully removed from the body and mounted in a series on glass slides. By means of a dissecting microscope of low power and a camera lucida the image of the wing was projected upon a magnified scale and the length thus read to tenths of a millimeter. With museum material it was necessary to measure the wings in situ and this was accomplished by the use of a metal scale divided to fifths of a millimeter placed against the wing under a lens.

The Frequency Polygons.

The size of a class was fixed at one fifth of a millimeter and this gave a range of ten classes. The polygon is bimodal, one mode being at 1.5 mm. and the other at 2.7 mm. The extremes of the range include from 1 mm. to 2.99 mm.

For convenience in calculation the polygon was considered as two, the first having six and the other five classes, the small connecting class being divided between the two polygons. Both polygons are skew, running down very rapidly on their outer slopes and shading off gradually toward each other to be connected by a very small class. The skewness of the polygon with the mode at 1.5 mm. is + .0235 and that of the one with the mode at 2.7 mm. is — .018.

An examination of short-winged specimens from California and from Long Island kindly loaned from the National Museum by Dr. L. O. Howard and others from New York State loaned by Dr. C. E. Felt gave polygons with the same mode as that obtained from short-winged material from Ohio. Similar results were obtained by a comparative study of long-winged insects sent from Urbana, Ill., by Professor S. A. Forbes. This indicates that the tendency of a given form is toward the same mode from whatever region taken or whether the two forms are mixed or separate.

Discussion of Results.

The significance of these results is by no means easy to determine. Looking at the polygons only it seems reasonable to suppose that the present dimorphic species has been derived from a parent stock with a mode lying somewhere between the two present ones. In that case it may be assumed that differences of environment have permanently impressed themselves, dividing the parent stock into two evolutionary lines one of which at present has wings longer and the other wings shorter than the parent stock.

The evidences of geographic distribution appear to negative this view. The genus is almost cosmopolitan, having been reported from every continent save Asia and from many islands of the sea. So far as known, it is most abundant and certainly most destructive in the United States. Nevertheless there are good reasons for regarding the chinch bug not as a native but as an immigrant. In his very reasonable hypothesis about the origin and distribution of the chinch bug in North America, Professor Webster (1898)[1] assumes that our stock of chinch bugs has come from South America by way of the Isthmus of Panama, Central America and Mexico. The north-flowing stream was divided first by the Cordilleran system, one branch of the division following the Pacific Coast northward, the other, by far the more important one, spreading over the Gulf States, was split again by the Appalachian Mountain System. One of these latter branches overflowed the Mississippi Valley; the other, following the coast of the Atlantic, finally rounded the north end of the mountain system and finding a congenial highway across New York State

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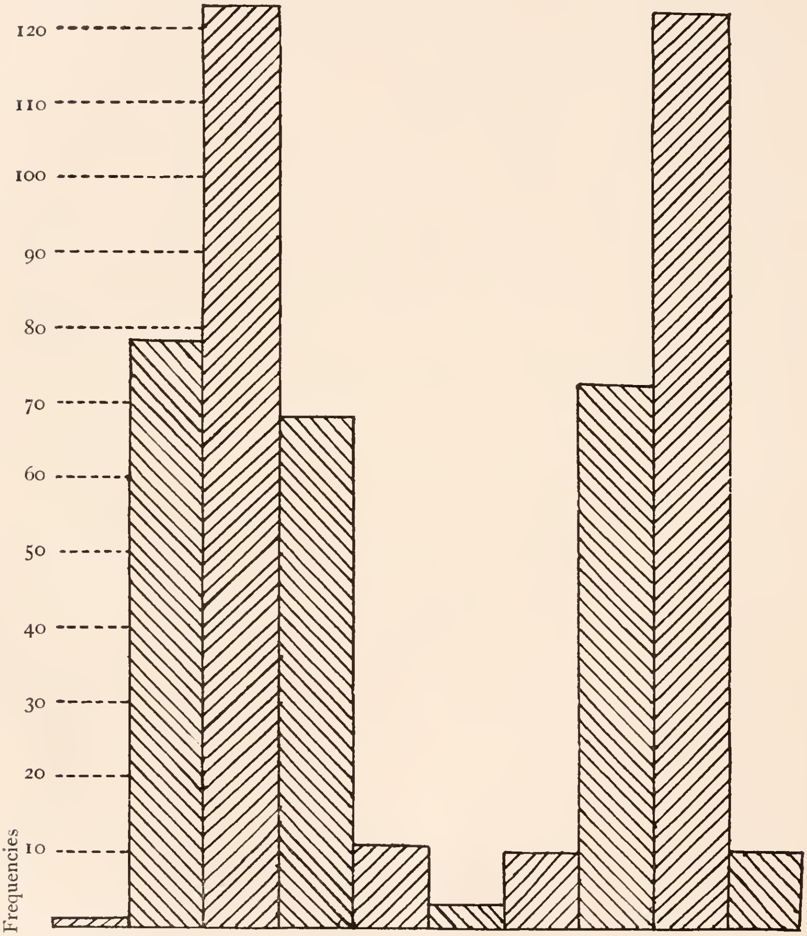
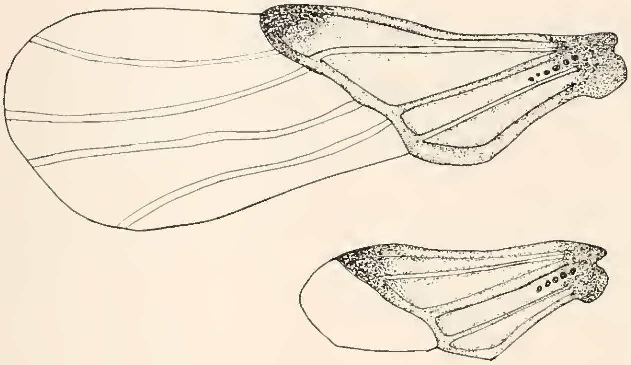
[1] Webster, F. M., "The Chinch Bug," U. S. Dept. of Agriculture, Bulletin No. 15, New Series.



joined the Mississippi Valley branch in northern Ohio and around the Great Lakes.

The short-winged form, so far as known in America, is confined to the ocean coasts and the immediate vicinity of the Great Lakes. The vast interior region from Central America to Manitoba abounds with only the long-winged form.

If Webster's theory is the correct one, we can scarcely escape the conclusion that the short-winged form originated in the regions where it is at present found. No short-winged specimens have ever been reported from the Gulf States outside of Florida, from Mexico or Central America, nor west of the Alleghanies, notwithstanding, the insect is common in those regions and the short-winged form has been carefully looked for in some of them. The long-winged insects, then, appear to have been the ancestral form in America as far as history and hypothesis can give a clue. There seems to be an inherent tendency in the species to produce the short-winged form when the proper ecological conditions are provided. How the species acquired this tendency is a very difficult thing to understand and it is not the purpose of this paper to attempt an explanation of a phenomenon that appears to be older than the division of Heteroptera into the present recognized families.



According to Saunders[1] dimorphism is exceedingly common among British Heteroptera and this caused much confusion because long- and short-winged forms were placed in separate species, certain other correlated characters, *e. g.*, a weaker developed pronotum in the short-winged form being constant.

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[1] Saunders, Edward, F.L.S., "The Hemiptera — Heteroptera of the British Islands," 1892.

In the Family Lygeidae all grades of winged shortening occur and in some species a fully-winged individual is very rare. Indeed every important family shows wings shortening to some extent.

Though the short-winged form occurs in America almost exclusively near large bodies of water such proximity is not necessarily a factor in producing and preserving this peculiar character.

A closely related species, *B. doriæ*, is comparatively abundant in southern Europe and far northward into the interior of Hungary. A long-winged specimen of this species is a rarity and was not supposed to exist until 1880 when a very small colony was discovered by Professor Sajö. From his paper, presented in full in Professor Webster's bulletin previously cited, we get the facts concerning this species.

The colonies of *B. doriæ* live on the bases of bushy grass near or even under the surface of the ground, and here the stages of development are passed through. The species is very widely distributed on sand drifts and in hilly regions, but long-winged specimens were found in but a single tiny spot. The bunches of grass on which the insect lives are isolated in partially bare ground. During the period of development, *great drought prevails.* The long-winged specimens possess a stronger and broader thorax than the short-winged ones, and it never attacks cultivated crops.

According to numerous observers cited by Professor Webster, the habits of *B. leucopterus* along our coasts are almost identical with those described for *B. doriæ.* Professor C. W. Woodworth writes me that the chinch bug is found in California chiefly in the salt marshes.

SUMMARY.

Where short-winged chinch bugs occur in Europe and America their habitat almost without exception compels them to live about the roots of tufts of grass on a soil otherwise almost bare. In California they are found in salt marshes. In Europe it may be added that the developmental stages occur at a season of great drought. Taken all together we have a picture *par excellence* of a xerophilous insect which is only another way of designating a species capable of withstanding hard or unfavorable conditions of living. Among the hard conditions which are responsible for dwarfed wings as well as more or less dwarfed bodies of chinch bugs, I should place first drought and poor food supply. Latitude and climate do not influence them, but edaphic conditions that may extend over large areas are the potent factors.

The only recorded observation that seems to oppose this view is that of Mr. E. P. Van Duzee.[1] He states that in portions of Ontario and New York where the short-winged form usually predominates, in dry, hot summers they mostly acquire fully developed wings. It seems possible, however, that a dry hot summer added to an ordinarily unfavorable habitat may have destroyed the short-winged form to an extent, only those in the most favored places being allowed to develop.

That the short-winged form should extend at times beyond the borders of the particular habitat which served to develop the dimorphic tendency (as occurs for example in northern Ohio) may be regarded only as the persistence for a time of a character acquired by the race even when the insect is in different surroundings. The mixed forms, however, always cling to old food habits as far as possible, taking by preference to grass meadows instead of attacking grain fields as do the long-winged insects of the interior.

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[1] Van Duzee, E. P., *Canadian Entomologist*, Vol. XVII., pp. 209–210, 1886.

UNIVERSITY OF CHICAGO,
June, 1903.

ON TWO CASES OF MUSCULAR ABNORMALITY IN THE CAT.[1]

RAYMOND PEARL.

The muscular anomalies here described were found by the writer in specimens of the domestic cat used for dissection in class work in the University of Michigan. As both of the cases presented certain interesting features it seemed advisable to publish an account of them at this time.

I. A Case of Abnormal Insertion of the M. Latissimus Dorsi.

In the cat the tendon of insertion of the M. latissimus dorsi normally is in two parts. One of these parts is joined by the muscle and tendon fibers of the M. teres major, and the conjoined tendon of these two muscles is inserted on the medial side of the shaft of the humerus. The other portion of the latissimus tendon, which may not be always present according to Reighard and Jennings,[2] joins with the pectoralis minor, reaching the bone along the line of insertion of the pectoralis minor. This line is along almost exactly the middle of the ventral face of the humerus. As a consequence of the existence of their different lines of insertion the two portions of the latissimus tendon form an arch, which makes up a part of the bicipital arch.

In a well-formed, adult male cat dissected by the writer the very peculiar arrangement at the insertion end of the M. latissimus dorsi shown in Fig. 1 was found on both sides of the body. From the cranial border of the latissimus a slip (Fig. 1, *x*), about 4 cms. long and 6 mm. wide passed craniad above that portion of the latissimus which joins the pectoralis minor (Fig. 1, *y*). This slip was inserted by fleshy fibers on the surface of the M. pectoantibrachialis on the medial surface of the leg, just beneath the skin. This band of muscle formed a very distinct, rather thick slip.[1] The relations of all the other muscles of the leg were normal. The two tendons of insertion normal to the latissimus dorsi were present and in their usual relations. The abnormal slip was simply added on, as it were, to the muscles normally present.

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[1] Contributions from the Zoölogical Laboratory of the University of Michigan, No. 65.

[2] Reighard, J., and H. S. Jennings, "Anatomy of the Cat." New York, 1901 p. 121.

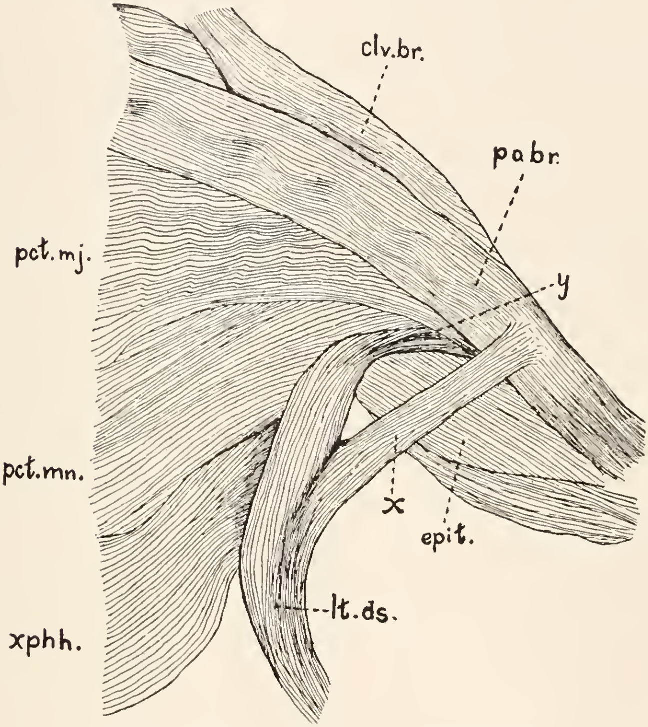

FIG. I. Ventral view of left side of the thoracic region in cat, showing abnormal insertion of the M. latissimus dorsi. *clv. br.*, M. clavobrachialis; *pabr.*, M. pectoantibrachialis; *epit.*, M. epitrochlearis; *lt. ds.*, M. latissimus dorsi; *xphh.*, M. xiphihumeralis; *pct. mj.*, M. pectoralis major; *pct. mn.*, M. pectoralis minor; *x*, abnormal slip of M. latissimus dorsi; *y*, portion of the latissimus dorsi which joins the pectoralis minor.

The conditions found in this case of the latissimus dorsi inserting in three portions, one of which does not reach the humerus at all, is apparently unique. So far as I have been able to discover no record of such a condition has been made in teratological literature, nor is such a condition found normally in any form. In most mammals[1] the latissimus inserts by one tendon; in some forms (*e. g.*, the cat) usually by two; and finally as a variation, which apparently occurs with some frequency, it inserts by two tendons in forms where it normally has only one. This last is the condition in man.[2]

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[1] In another cat dissected by a student in the laboratory precisely the same arrangement was found, except that the mucle slip was much thinner than in the case here described. Only a few fibers reached the pectoantibrachialis.

The condition found in this abnormality to a certain degree resembles morphologically what is normally found in many mammals in the M. epitrochlearis. This muscle, in the majority of cases, takes origin from the surface of the latissimus dorsi near its insertion, and is inserted into the superficial fascia of the forearm and the olecranon. This muscle is usually regarded as a differentiation product of the latissimus dorsi. It is possible that the present abnormality may indicate that originally the M. epitrochlearis had in the carnivora a greater extent at its insertion, extending on to the superficial fascia of the upper as well as the forearm. Further than this I am not able to make any suggestion regarding the significance of this abnormality. On account of the fact that apparently such a case had not been described, it seemed desirable to make a record of it.

II. A CASE OF CONNECTION BETWEEN THE M. CLEIDOMASTOIDEUS AND THE M. LEVATOR SCAPULÆ VENTRALIS.

The M. cleidomastoideus normally forms a distinct muscle in the cat, taking its origin from the apex and caudal margin of the mastoid process of the temporal bone. It passes caudad, flattening during its course, and is inserted on the lateral four fifths of the clavicle and laterad of the clavicle on the clavicular raphe. This clavicular raphe is formed between the Mm. cleidomastoideus and clavotrapezius (= M. cleido-occipitalis + cleido-cervicalis Streissler)[3] craniad, and the M. clavobrachialis (= Pars claviculi of M. deltoideus of earlier writers) caudoventrad. At its insertion the cleidomastoid lies entirely beneath the clavotrapezius. Lying close besides the cleidomastoid (dorsad and in part mediad) is the M. levator scapulæ ventralis (= M. omo-transversarius Streissler, *loc. cit.*, = Pars ventralis of the M. omo-cleidotransversarius Leche, *loc. cit.*, = "omo-trachélien" Le Double, *loc. cit.*). This muscle in the cat takes origin by two heads, one coming from the basis cranii opposite the middle of the bulla tympani, and the other from the ventral surface of the transverse process of the atlas.

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[1] Cf. Leche, W., Mammalia, in Bronn's "Klassen u. Ordnungen des Thier-Reichs," Bd. 6, V. Abth., 1874–1900, pp. 722–725.

[2] Cf. Le Double, A. F., "Traitè des Variations du Système Musculaire de l'Homme," Paris, 1897, T. I., pp. 194–202.

Testut, L., "Les Anomalies Musculaires chez l'Homme," Paris, 1884, pp. 106–118.

[3] Streissler, E., "Zur vergleichenden Anatomie des M. cucullaris und M. sternocleidomastoideus," *Arch. f. Anat.* (*u. Physiol.*) *Jahrg.*, 1900, pp. 335–365, Taf. XXI. u. XXII.

In a well-developed adult female cat dissected by the writer, the following abnormal relation of the cleidomastoid and the levator scapulæ ventralis was found on the left side of the body. At almost precisely the middle point of the levator scapulæ ventralis a thick muscle band, approximately 4 mm. wide, passed from the ventral border of this muscle cranioventrad to the dorsal border of the M. cleidomastoideus, with which muscle it joined. The connecting band was throughout its length of approximately the same thickness as the Mm. cleidomastoideus and levator scapulæ ventralis at the places where it joined them.

In considering the significance of this abnormality the possibility of its representing a case of reversion may be dismissed at once, because in their comparative anatomy the cleidomastoid and levator scapulæ ventralis are known to be quite distinct muscles. The M. cleidomastoideus is a differentiation from the general sternocleidomastoid group of muscles, which in turn is to be considered as having separated from the trapezius group.[1] It belongs to the rather thin, superficial sheet of muscle which covers the dorsal, lateral and part of the ventral surface of the neck, and the dorsal surface of the cranial thoracic region in all the Mammalia. This sheet of muscle breaks up into varying numbers of separate muscles in different groups. All of these muscles, however, as has been very clearly brought out by Streissler (*loc. cit.*), fall into either a dorsal or a ventral group. The dorsal group may be characterized as the dorso-scapularis-trapezius group, and the ventral as the sternocleidomastoid group. All the muscles of this superficial layer are innervated primarily by the N. accessorius, with, in some cases, fibers from the cervical plexus going to the muscles of the ventral group. The levator scapulæ ventralis or omo-cleido-transversarius, pars ventralis (Leche) belongs to an entirely different set of muscles than those just considered. According to Leche[1] it is highly probable that this muscle is a differentiation product of the muscle group from which the M. levator scapulæ comes. It is innervated by fibers from the ventral branches of the spinal nerves.

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[1] Cf. Leche, *loc. cit.*, pp. 701–706.

Evidently then, since the cleidomastoid and the levator scapulæ ventralis have such different sources the abnormality under discussion cannot be considered as a reversion.

The abnormality does, however, seem to be suggestive as possibly giving us light on the meaning of the conditions found in man with reference to the muscles of the ventral neck region. In what manner will be apparent if the relations in man are considered briefly. The M. omotransversarius (*i. e.*, levator scapulæ ventralis) is normally found in some form or other in practically all mammals up to man. In man it is only occasionally present as a separate muscle in abnormal cases. It has been a problem how to account for the absence of this muscle under normal conditions in man, and no satisfactory explanation for it has ever appeared so far as is known to the writer. On the other hand the human sternocleidomastoid is, of course, a complex muscle, made up by the fusion of elements normally forming distinct and separate muscles in the lower forms. Streissler[2] has shown that this muscle contains at least the following elements: In the superficial portion a sternomastoideus superficialis, a sterno-occipitalis and a cleido-occipitalis element; and in the deep layer a sternomastoideus profundus and a cleidomastoideus element.

The fact that occasionally the omotransversarius appears in man as a distinct muscle may be taken as strong presumptive evidence that in all cases the muscle is present in man as an element in the ventral neck musculature. Why it is not found under normal circumstances is because it is indistinguishably fused with some other muscle. In the abnormal cases where it does appear as a separate muscle we most probably have simply a failure to fuse or only partial fusion, where normally complete fusion occurs.

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[1] *Loc. cit.*, pp. 731–735.

[2] *Loc. cit.*

The abnormality here under consideration has suggested to me the view that *normally in man the omotransversarius element is fused completely with the cleidomastoid portion of the M. sternocleidomastoideus.* This view would make the sternocleidomastoid a complex of six elements, as shown in the following scheme:

M. sternocleidomastoideus (Man)	Sternomastoideus superficialis Sterno-occipitalis Cleido-occipitalis	Superficial.
	Sternomastoideus profundus Cleidomastoideus *Omotransversarius*	Deep.[1]

The evidence for this view comes from two sources. In the first place, the occurrence in anomalous cases in man of a separate M. omotransversarius makes it extremely probable that this element is generally present in man, but in normal cadavers is completely fused with some other muscle. In the second place, the anomalous case in the cat just described shows that in a form lower than man it is possible for a partial fusion of the cleidomastoid and omotransversarius muscles to occur as a variation. This makes it seem probable that the muscle complex with which this omotransversarius element in man normally fuses is the sternocleidomastoid.

SUMMARY.

1. A case of insertion of a portion of the M. latissimus dorsi on the M. pectoantibrachialis is described.

2. A case of partial union of the Mm. cleidomastoideus and levator scapulæ ventralis (or omotransversarius) is described.

3. The view is advanced that the human sternocleidomastoid muscle contains an omotransversarius element. This element is normally completely fused with the deep portion of the sternocleidomastoid, but, in abnormal cases, it may fail to fuse completely and consequently then appears as a separate muscle.

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